THE REMOVAL OF MICROBIAL AND CHEMICAL CONTAMINANTS FROM AQUEOUS SOLUTIONS BY PARTICLES COATED WITH IRON AND ALUMINUM HYDROXY(OXIDES).

By

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As with my thesis, I dedicate this dissertation to my beloved mother, Paullette Azzu; God bless her soul. Her spirit has lighted my path during the darkest of times. Many times I pleaded for her guidance and help, and I have always felt as if she was there to help me overcome my hardships.

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TABLE OF CONTENTS

ACKNOWLEDGMENTS iii

LIST OF TABLES ix

LIST OF FIGURES xi

ABSTRACT xiii

CHAPTERS

1. INTRODUCTION 1

Waterborne Diseases and their Etiological Agents 1
Bacterial Diseases 3
Protozoan Diseases 3
Viral Diseases 4

Waste and Drinking Water Treatment Processes and Public Concern .. 5

Mechanisms Involved in the Adsorption of Microorganisms to Solids .. 10
Bacterial Adsorption 11
Viral Adsorption 12

Improving Filtration Media 13

Need for Improving Virus Capture and Detection 16

Potential Uses of Improved Filter Media 17

Purpose of this Study 19

2. MODIFICATION AND CHARACTERIZATION OF THE SOLIDS 22

Materials and Methods 25

Modification of Sand and Diatomaceous Earth 25
Metal Content of Modified Particles and Filter Effluents 27
Coating Durability Tests 27
Zeta Potential Measurements 28
Scanning Electron Microscopy 28
Statistical Analysis 28

Results 29
Metal Content of Metal Hydroxide Coated Solids 29
Coating Durability Tests. 29
Zeta Potential 30
Scanning Electron Microscopy 30

Discussion 31

3. REMOVAL OF VIRUSES FROM AQUEOUS SOLUTIONS BY COLUMNS OR FILTERS CONTAINING MODIFIED PARTICLES 40

Materials and Methods 44
Modification of Sand 44
Bacteria 46
Detection of Bacteriophages and Enteric Viruses by Standard Methods 46
Detection of Enteroviruses by RT-PCR 47
Viable Enterovirus Detection by IC/RT-PCR 49
Detection of Rotaviruses by RT-PCR 49
Protozoan Parasites 50
Detection of Cryptosporadium parvum by PCR, RT-PCR, and Cell Culture Integrated PCR 51
Removal of Microorganisms by Sand Coated according to Various Methods 52
Large 100 mesh Sand Column Setup and Viral Removals 53
35 mesh Sand Column Filter Setup and Viral Removal 54
Swimming Pool Filter Setup and Microorganism Removals 55
Tap Water Facet Filter Setup and Microorganism Removals 56
Statistical Analysis 57
Water Metal Analysis 57

Results 58
Removal of Microrganisms by Sand Coated According to Various Methods 58
Removal of Microorganisms by Large 100 Mesh Sand Columns 58
Removal of Microorganisms by Columns Containing Coated 35 Mesh Sand 59
Removal of Microorganisms by Swimming Pool Filters 60
Virus Removals by Tap Water Faucet Filters Containing Coated Solids 61
Water Metal Analysis 62

Discussion 62

4. THE EFFECT OF SALTS ON THE ADSORPTION OF VIRUSES TO COATED SOLIDS, UNCOATED SOLIDS, AND MICROPOROUS FILTERS 83

Materials and methods 85
Viruses 85
Microporous Filters 85
Chemical Solutions 86
Aluminum Titration Experiments 86
Experimental Procedure 86

Results 88
Effect on Salts on the Adsorption of Viruses to Microporous Filters 88
Effect of Salts on the Adsorption of Viruses to Coated or Uncoated D.E. 90

Discussion 91
Effect on Salts on the Adsorption of Viruses to Microporous Filters 91
Hydrophobic Interactions 92
Indirect Effects 93
Viruses and Filters Studied 94
Effect of Salts on the Adsorption of Viruses to Coated or Uncoated D.E. 95

5. REMOVAL OF CHEMICAL CONTAMINANTS FROM AQUEOUS SOLUTIONS ... 108

Materials and Methods ... 110
Modification of the Sand ... 110
Column Adsorption Experiments ... 111
Synthetic Sewage Solutions ... 111
Chemical Solutions ... 112
Column Adsorption Experiments ... 112
Batch Adsorption Experiments ... 113
Complex Organic Mater and Dissolved Organic Carbon (DOC) Removals Metal Analysis ... 113
Protein Removal Experiments ... 113
Metal Analysis ... 114
Sand Metal Content Analysis ... 114
Phosphate, Nitrate, and DOC Analysis ... 114
Statistical Analysis ... 115

Results ... 115

Discussion ... 117

6. CONCLUSION ... 124

LIST OF REFERENCES ... 127

BIOGRAPHICAL SKETCH ... 135

LIST OF TABLES

Table		page
1.	Major infectious agents present in inadequately treated drinking water worldwide	21
2.	Amount of iron and aluminum deposited on the coated sand as determined by ICAP of the digestate	37
3.	The effect of attritioning on the metal content of 35 mesh sand coated by different methods.	38
4.	The Zeta potential of 35 mesh sand or D.E. coated according to different methods.	39
5.	Sequences of the primers used for the detection of enteric viruses	76
6.	Removal of viruses by 35 mesh sand coated according to the different methods before and after attritioning	77
7.	Comparison of RT-PCR and cell culture for determining the removal of rotavirus from water by columns containing coated or uncoated 100 mesh sand Removal of viruses from water by columns containing coated (method 2) or uncoated 35 mesh sand	78
8.	Removal of viruses from water by columns containing coated (method 2) or uncoated 35 mesh sand	79
9.	RT-PCR and IC/RT-PCR of dilutions of water seeded with enteric viruses and recirculated through the columns containing coated (method 2) or uncoated 35 mesh sand	80
10.	Removal of Cryptosporidium parvum by 35 mesh sand columns according to PCR, RT-PCR, and cell culture integrated	81
11.	Removal of microorganisms by Porpoise filters containing modified or unmodified sand.	82

12. Influence of salts on virus adsorption to microporous filters at pH 7. . . . 97

13. Influence of salts on virus adsorption to microporous filters at pH 3.5 . . 98

14. Influence of 0.1 M $AlCl_3$ or 0.1 M $MgCl_3$ and 4 M urea on virus adsorption to microporous filters at pH 7 and 3.5 99

15. Effect of salts and urea (or 0.1%Tween 80) on viral adsorption at pH 3.5 100

16. The effect of prefiltering the aluminum solutions used in viral adsorption experiments to Millipore HA filters at pH 7. 101

17. The influence of salts and urea on the adsorption of viruses to coated and uncoated diatomaceous earth at pH 7 and 3.5 102

18. The composition of synthetic waste water used in column and batch challenge tests (mg/l). 119

19. Removal of chemical contaminants from water or simulated sewage by columns containing coated or uncoated sand 120

20. The metal content of sand at the beginning, 1 day, and 1 week after challenge with simulated sewage containing salts of the indicated metals 121

21. Batch removal of chemical contaminants from water or simulated sewage by coated or uncoated sand 122

LIST OF FIGURES

Figure page

1. Schematic of the developed particle coater used in method 4 34

2. Scanning electron micrograph of the surface of sand coated according to method 2 35

3. Electron microscope XPS elemental analysis of sand coated with ferric and aluminum 36

4. Schematic of the column filter set up used in viral removal experiments .. 68

5. Schematic of the swimming pool filter setup described in the Materials and Methods Section. 69

6. Schematic of the faucet filter set up used in viral removal experiments .. 70

7. Removal of viruses by columns (35x5 cm) containing 1 kg of 100 mesh sand coated according to method 2 71

8. Removal of viruses by columns (35x5 cm) containing 1 kg of 100 mesh uncoated sand 72

9. Removal of viruses from water by swimming Pool Filters containing Modified or Unmodified sand 73

10. Removal of Microorganisms by faucet filters containing coated solids. ... 74

11. Scanning electron micrograph of the surface of coated sand that had water passed through it for 30 days. 75

12. A schematic of the setup used to contain the diatomaceous earth for the adsorption interference experiments 103

13. The effect of magnesium chloride concentration at pH 7 on the removal of polio 1 virus by 1 MDS and Millipore HA filters. 104

14. The effect of magnesium chloride concentration at pH 3.5 on the removal of polio 1 virus by 1 MDS and Millipore HA filters 105

15. The effect of aluminum chloride concentration and the ph on the adsorption of polio 1 virus to Millipore HA filters 106

16. The effect of magnesium chloride concentration on the adsorption of polio 1 virus to Millipore HA filters an 1 MDS filters. 107

17. The removal of chemical contaminants from water by columns containing coated sand. 123

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REMOVAL OF WATERBORNE MICROBIAL AND CHEMICAL CONTAMINANTS BY METALLIC HYDROXY(OXIDE) COATED SOLIDS

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A process for coating solids with precipitates of metallic precipitates was developed. Salts of aluminum and iron were used to produce the corresponding hydroxy(oxide) coating. Diatomaceous earth and sand were efficiently coated by this process. The coating process deposited significant amounts of metal onto the particles. The resulting coatings were more continuous, durable, and electropositive than other previously described coatings. Coated particles efficiently adsorbed microorganisms in water. Particles coated according to this method efficiently removed a variety of waterborne pathogens from test solutions. Bacteriophages (MS-2, PRD-1, and ϕX-174), Bacteria (*Streptococcus faecalis* (ATCC 19433), *Staphylococcus aureus* (ATCC 12600), *Salmonella typhimurium* (ATCC 19585), and *Escherichia coli* (ATCC 15597)), enteroviruses (Polio 1, Echo 1, Echo 3, Coxsackie B 3, Coxsackie B 5, and rotavirus SA 11)), and protozoan parasites (*Cryptosporadium parvum* oocysts) were used to challenge the filters containing the coated solids. Coated sand or

diatomaceous earth was placed in columns, swimming pool filters, and faucet filters. The solids increased the microbial filtration efficiency of these filter units by several folds. The coated solids maintained their ability to remove microorganisms from water for extended periods of time when they were incorporated in water filtration units. At all times, coated solids outperformed uncoated ones in removing a variety of microorganisms. Adsorption of viruses to the modified particles was determined to be mainly electrostatic in nature. Various procedures for determining the removal and viability of the enteric viruses and protozoan parasites were evaluated. Integrated cell culture followed by reverse transcriptase/polymerase chain reaction (ICC/RT-PCR) amplification of conserved genomic sequences proved to be the most sensitive and least time consuming in determining the presence of infectious pathogens. The detection of pathogens by RT-PCR of viral (or PCR of protozoan) genomic sequences alone was insufficient for the determining of the presence of the infectious microorganism. In addition to removing microbial contaminants, the coated solids efficiently removed a variety of added chemical contaminants from water or simulated sewage solutions. They effectively removed heavy metals, phosphates, metals, non organic materials, and proteins. At all times, ferric and aluminum ions were not detected in all filter effluents. The enhanced adsorptive behavior of these modified particles combined with their stability and low-cost renders them superb filter media for the removal of a variety of water contaminants.

CHAPTER 1
INTRODUCTION

Waterborne Diseases and Their Etiological Agents

Waterborne disease cases and outbreaks are prevalent worldwide. They constitute a major public health dilemma in both developed and developing countries (Payment et al., 1991a; 1991b; Rao, 1976). The Pan American Health Organization estimates that 40 percent of the homes in the earth's southern hemisphere have inadequately treated or unsafe water (Epstein, 1996). Poor water quality is one of the foremost causes of disease and death in these countries. In developing countries at least half of the population is affected by waterborne diseases. This breadth of this problem extends to many developed countries. A recent report by the Academy of Microbiology indicated that our surface and drinking waters are no longer microbiologicaly safe (Colwell, 1996). According to the report, there has been a significant rise in waterborne diseases worldwide.

In the United States, public concern over the quality and safety of our drinking water has been rising. A recent article that appeared in the national newspaper USA Today warns the public of the dangers associated with using

tap water (Eisler et al., 1998). According to the article, millions of Americans receive water that contains dangerous contaminants exceeding the legal limits. In addition, millions also get water that had not been tested properly, so there is no measure of its quality. Numerous people get sick everyday. Some actually die. The annual social costs of the reported waterborne mild gastrointestinal illnesses in the United States alone has been estimated to be over 22 billion dollars. The average yearly disease cases reported in the United States that were attributed to contact with waterborne pathogens were estimated to be at a low of one million and a high of seven million (ENEWS staff, 1998). The deaths were estimated to be between 1000 and 1200 per year. From 1986 to 1988, 50 waterborne disease outbreaks with 25,846 cases of disease were reported in the United States (Levine et al., 1991). Between 1991 and 1992, 17 states and territories in the United States reported 34 outbreaks associated with drinking water. These outbreaks caused an estimated 17,464 persons to become ill (Moore et al., 1993). In 1993, a waterborne outbreak of Cryptosporidiosis occurred in Milwaukee. During this outbreak over 400,000 people had intestinal disease (Morbidity and Mortality Weekly Report, 1995). The most common illness of water associated diseases is gastroenteritis; other illnesses include hepatitis, typhoid fever, mycobacteriosis, pneumonia, and dermatitis (Levine et al., 1991; Payment et al., 1994; Sherris et al., 1990). Waterborne gastroenteritis is an important public health problem in both developed and developing countries (Zhou et al.,1991). However, their prevalence is much more common (and less documented) in developing and under developed countries.

The pathogens that have caused these outbreaks include viruses, protozoa, bacteria, and helminths. Table 1 provides a list of the most common infectious agents present in contaminated waters worldwide (Geldreich, 1996). A summary of the data of Craun (1986) and Smith (1993) shows that viral agents were usually responsible for about 17% of these outbreaks, protozoans for about 30%, and bacteria for about 13%, while chemical poisoning accounted for about 6%. However, in many outbreaks of waterborne diseases, no infectious agent could be identified (Moore et al., 1993). Craun (1986), Moore et al. (1993), and Smith (1993) reported that no causative agent was identified in approximately 35% of the outbreaks.

Bacterial Diseases

Several species of bacteria may be associated with waterborne diseases. Incidents of waterborne disease outbreaks have been associated with enterotoxigenic *E. Coli, Camphylobacter jejuni, Vibrio cholerae, Salmonella* spp., (including *S. typhi), Yersinia enterocolitica* and *Shigella* spp. These have been reported in both developing and developed countries (Craun, 1986, Echeverria et al. 1981). As recently as July 4th 1998, a waterborne *E. coli* outbreak was reported in the city of Alpine in Wyoming (Eisler et al., 1998). More than 250 people suffered from intestinal diseases and many were hospitalized.

Protozoan Diseases

Other worldwide major water contaminants are the protozoan parasites. During 1986-1988 protozoan parasites caused 10 of 50 waterborne disease

outbreaks. *Giardia* caused nine of them and *Cryptosporadium* caused one. Of the 25,846 waterborne disease cases in that period, 1,169 were caused by *Giardia* and 13,000 were caused by *Cryptosporadium* (Smith, 1993). The largest waterborne outbreak occurred in 1993. That year, an outbreak of Cryptosporidiosis affected more than 400,000 persons in Milwaukee. This outbreak was associated with water obtained from a municipal water plant that was operating within the existing federal and state regulations. In the United States, all outbreaks of waterborne cryptosporidiosis detected between 1984-993 occurred in areas where water treatment procedures met state and federal standards (MMWR, 1995). In addition to causing these outbreaks, waterborne protozoan parasites caused numerous cases of gastroenteritis and other parasitic infection worldwide every year (Sherris et al., 1990).

Viral Diseases

There are more than 100 different enteric viruses excreted with feces that could be potentially transmitted by water. Members of this group include hepatitis viruses, rotaviruses, echo viruses, coxsackie viruses, polio, and Norwalk viruses, all of which have been implicated in outbreaks of waterborne disease (Gerba 1984; Gerba et al., 1988; Sherris et al., 1990). Gerba and Smith (1982) reported the detection of more than 374 pfu/liter of rotaviruses and more than 120 pfu/liter of enteroviruses in final treated effluent from a water treatment plant. Other workers had reported the detection of hepatitis A viruses in final water treatment effluents (Dvizia et al., 1993).

It is more than likely that previously unidentified viruses caused many of the outbreaks that had no reported pathogenic agent associated with them. Therefore, the actual number of viral associated water borne outbreaks might be a lot higher (Craun,1986; Seeley and Primrose, 1979). In the recent years, several newly discovered pathogens (mostly viral) have been identified and shown to be transmitted by water. These include Norwalk and related viruses, enteric hepatitis E, and Adenovirus 40/41 (Murray et al.,1996).

Besides causing outbreaks, waterborne pathogens may contribute to the background level of gastroenteritis. In an epidemiological study of drinking water related gastrointestinal illnesses in Canada during 1988-1989, Payment et al. found that tap water treated by conventional methods and meeting current water quality requirements still caused 35% more of the gastrointestinal disease symptoms than the water treated by advanced reverse osmosis units (Payment et al., 1991a; 1991b).

In conclusion, previous incidents and data show that water transmission is an important route of infection for humans (Smith 1993).

Waste and Drinking Water Treatment Processes and Public Concern

The incidence of waterborne disease outbreaks has been greatly reduced in developed countries where waste water and drinking water are extensively treated. Concerns about the level of pathogens in treated drinking and waste water have forced municipalities and government agencies to apply and enforce

strict standards on the quality of the final water effluent. In 1989, United States Environmental Protection Agency promulgated the surface water treatment rule. This rule mandated that water treatment plant processes achieve an overall 3 log reduction in the number of Giardia and Cryptosporadium, and a four log reduction in the number of enteric viruses. Subsequent amendments to the rule declared that large water treatment plants must produce an effluent with a maximum contaminant level goal (MCLG) of zero for *giardia, Legionella, cryptosporadium*, and viruses. This regulation also established a treatment technique requirement for dealing with these problematic contaminants (USEPA, 1998). According to this rule effluents must contain less than 1 virus or oocyst per 1,000 liters of water. At the same time the EPA also declared the total coliform rule that mandated the same requirement for fecal coliform bacteria in plant effluents. Since then, the EPA has implemented even more strict addenda to this rule and set regulatory guidelines for many of the water treatment processes. Similar rules are being implemented worldwide.

The strict water quality guidelines set by state and government agencies have forced water plants to resort to advanced filtration methodologies and extensive disinfection practices. Disinfection is commonly used as the final step of treatment and usually involves either the addition of strong bactericidal agents to the water or the exposure of the water to physical disinfection. Strong oxidizing agents such as chlorine, bromine, ozone, and others are commonly used disinfectants in water treatment. The low cost and effectiveness of chlorine have made it the most popular disinfectant in the water treatment industry for the

past decades. However, the effectiveness of chlorine is limited when it comes to the the inactivation of the Cryptosporadium oocysts in water. As our knowledge of the microorganisms increases, we start to recognize the limitations of many of the previously heavily relied on disinfection processes. In addition to these shortcomings, many disinfectants may react with the organic matter present in the water to form toxic and harmful byproducts (trihalomethane compounds, THMs). These are then released into the environment and possibly consumed by humans; they cause much environmental pollution and especially ground water pollution (Urano et al., 1991; WHO, 1984). These byproducts have been shown to be carcinogenic and have been linked to a variety of diseases. Much debate has focused on the use of these disinfectants for water treatment, but all the parties agree that disinfection is necessary. Ozone has been suggested for use as a safer alternative for chlorination. However, Chan and Larson have reported on the production of mutagens from the treatment of waste water with ozone (Chan and Larson, 1991). Many disinfection processes have been suggested and their use is currently being extensively examined.

As mentioned, in addition to the biological concerns associated with drinking water, chemical concerns are also very important. In recent years, many government agencies have enforced stricter quality standards and monitoring procedures for water plants to follow and imposed stiffer penalties for those who do not. For example, the Environmental Protection Agency has issued the Information Collection Rule that requires all water utilities to test for the different pathogens associated with waterborne diseases and for harmful

chemicals in the final treated water. This rule will help in establishing new treatment and disinfection standards and force water municipalities to improve treatment processes and ultimately their final water quality.

In the past, potable water sources were regarded as an unlimited and replenishable resource. Little attention was directed toward the problems associated with their consumption or contamination. The past and current tremendous population growth, agricultural advances, and industrial and urban expansion have directed much attention to the problem of water pollution. Potable water sources are being exhausted all around the world and especially in underdeveloped or developing countries (Pickford, 1990). This increased pressure has led to the search of alternative water sources and procedures for increasing the quality of current non-potable water resources.

The domestic use of reclaimed water has gained much interest in the recent years. Its use for domestic purposes is being extensively studied. Currently, reclaimed water is gaining acceptance for use in non-crop irrigation and aquifer recharging. Additional uses are being considered and debated everyday. However before such considerations take effect, water reclamation facilities must improve on their treatment processes and/or employ stringent monitoring protocols to ensure the safety and quality of their final treated effluent. The thought of using reclaimed water for domestic purposes alone has raised much public concern. This concern, combined with concerns over the previous numerous drinking water disease outbreaks, has already led many treatment plants to increase measures in water treatment, employ additional

filtration steps, upgrade existing systems, and/or use higher concentrations of potentially harmful disinfectants.

The numerous concerns about water quality and its implications on our health and environment have forced researchers and water management districts to search for better and safer methods for water treatment and/or to improve on current methods. Deep bed filtration through granular material is commonly used to remove particulates from water and thus improving the aesthetic and chemical quality of the water and reducing the need for and the amount of subsequent disinfection (Stenkamp, 1993). Water filtration using sand or diatomaceous earth has been used for many years in drinking and waste water treatment (Brown et al., 1974a; 1974b). The finer sized diatomaceous earth is a more efficient filtering medium. However, it costs more, requires more care, and is subject to clogging more frequently. Sand, on the other hand, is cheap, easier to handle, and lasts longer, but does not work as well. The process of filtration through these granular media mainly depends on the ability of these poorly adsorbing materials to mechanically remove microorganisms. Improving the adsorbent qualities of these materials would greatly enhance the filtration process. Much research and development have been dedicated to this aspect. For example, in slow trickling sand filters, biofilms are intentionally formed on the surfaces of sand particles to increase their adsorptive potential. This requires first running the filter until the biofilm has coated the filtering media, also known as ripening of filter media, then using it for filtration. This initial step may take up to a month to perform. However, the layer of microorganisms that forms on the

sand particles is efficient in the entrapment of floating microorganisms and suspended solids. Another sand filtration technique requires the use of added chemicals. In rapid sand filters, plain sand is used and inorganic or organic flocculants are added prior to filtration to aid in the removal of microorganisms. Periodically, the filter is back washed to avoid clogging and to rid of the trapped impurities. These inorganic flocculants (metallic oxide or hydroxide precipitates) or organic flocculants (cationic polymers) greatly adsorb many viruses and bacteria (Parviz and Engelbrecht, 1975). They have been used in drinking and waste water treatment for years to promote flocculation and increase filtration efficiency (Viessman and Hammer, 1985).

Mechanisms Involved in the Adsorption of Microorganism to Solids

Interactions between suspended particles and solids in aqueous solutions have been reported and described extensively in the literature (Daniels, 1980; Esterman and McLaren, 1959, Farrah,1982; Farrah et al. 1992; Gerba, 1984). Physical and chemical interactions are both involved in particle-particle interaction in aqueous environments. Electrostatic interactions resulting from the different charges exhibited on the particles surface influence the degree of attractive or repulsive forces between particles. Microorganisms, overall, have a net negative charge that results from the net charge of their surface chemical moieties. This net charge favors the adsorption of microorganisms to electropositive surfaces. Daniels (1980) reported that the

most determinant factor in the adsorption of microorganisms is the surface charge of the organisms and the charge on the solids. The presence of hydrophobic moieties on the particle's surface also determines the degree of association with other particles or surfaces in the aqueous environment. Microorganisms tend to have a variety of hydrophobic groups on their surfaces. This hydrophobic character renders their presence in the aqueous environment energetically unfavorable and causes their adsorption to other hydrophobic surfaces to reduce water tension. Many reactive surface groups also contribute to and influence a variety of bonding isotherms between these solids. Examples of these bonds would be hydrogen bonding, cation bridging, cation exchange, and double layer compression. In addition, the adsorption of microorganisms to solids has been shown to be dependent on several external factors, including the pH, temperature, cation concentration, and ionic strength of the surrounding medium (Daniels, 1980; Gerba, 1984). The understanding of these forces of interaction has led to the development of methods and apparatuses for the removal of microorganisms from water. An example of the practical application of these principles is the adsorption of microorganisms by the positively charged alum during waste water and drinking water treatment procedures.

Bacterial Adsorption

The interactions between bacterial cells suspended in a fluid and solid surfaces are dependent on the pH and ionic strength of the surrounding

medium. The strongest adsorption generally occurs at pH values close to the isoelectric point (pH3-6) of the microorganisms (Estermann and McLaren, 1959; Gunnison and Marshall, 1937; Daniels, 1980). The adsorption of bacterial cells that normally possess net negative charges to commonly found negative surfaces may be enhanced by the addition of multivalent cations or precipitates of these cations to the solution. In addition to net negative charge on bacterial surfaces, there is an abundance of hydrophobic moieties on the cell surface. Because of the complexity of the bacterial cell surface, the mechanisms of adsorption to solid surfaces involve many phenomena. An individual cell can participate in certain ion exchange bonds, form bonds with water soluble polyelectrolytes, and attach nonspecifically via hydrophobic or electrostatic interaction to corresponding surfaces.

Viral Adsorption

Virus particles at their simplest consist of a strand of nucleic acids surrounded by a protein coat. They range in size from 20-300 nanometers. In solution, they behave as amphoteric colloids with surface charges resulting from the ionization of surface groups (i.e. carboxyl, amino, etc.). The ionizations of these groups contribute to the net charge of the virus as a function of pH. The pH at which the virus has an overall zero charge is called the isoelectric point. At a pH below or above the isoelectric point, the virus exhibits a positive or negative charge, respectively.

Mix (1974) discussed the mechanisms involving virus adsorption and suggested several mechanisms that explain virus interactions with membranes. These include hydrophobic, hydrogen bonding, and electrostatic forces. Those interactions depend on the pH, salt concentration, and organic matter present in the water (Mix,1974; Bitton, 1980). In general, lowering the pH will increase viral adsorption. The presence of certain salts promote the association of viruses with membrane filters by strengthening the hydrophobic interactions (Farrah, 1982). Farrah et al. (1991) demonstrated that hydrophobic and electrostatic interactions were both essential in viral adsorption to membranes and biological flocs. However, the electrostatic interactions are thought to be the predominant force responsible for the viral adsorption to diatomaceous earth and sand. In addition, the presence of organic matter in the aqueous solutions interferes with viral adsorption to solids (Bitton and Mitchell, 1974; Gerba, 1984). Overall, these studies indicate that the different mechanisms of viral adsorption depend on the characteristics of the different materials used for adsorption or on the nature of the coatings on the surface of the solid.

Improving Filtration Media

Much attention has been devoted to the chemical modification of filter media. If the filtering media were to be modified to increase their filtration efficiency and aid them in adsorbing microorganisms, then these materials would be far more efficient than the conventional media used currently. The

filter media adsorption of microorganisms would lead to shorter ripening time (in rapid trickling filters) or a better removal efficiency (in rapid filters) or higher filter adsorption and detection efficiency (in viral concentration and recovery procedures). This could also lead to the decreased need for the use of potentially harmful disinfectants and provide extra insurance in case something does go wrong in the operation of the water plant.

Cationic polymers can be used to coat filtering materials to increase their filtration efficiency. Brown et al. (1974a; 1974b) found that diatomaceous earth coated with cationic polymers adsorbed more viruses than did untreated diatomaceous earth; however, the coating was unstable with time. Preston et al. (1988) found that many cationic polymers can be used to coat microporous filters and increase their ability to adsorb viruses from water. Zerda et al. (1985) studied the effects of the addition of positive charges to silica using organosilane derivative and documented improved filtration efficiencies also. However, cationic polymers and silanes may not be cost effective when used in large scale water treatments and they, or their break down products, may be toxic if were present in drinking water (Parviz and Engelbrecht, 1975). Brown et al. (1974a; 1974b) also found that coatings of ferric hydrates and aluminum hydrates increased viral adsorption. However, these precipitates adhered poorly and irregularly to sand and diatomaceous earth and came off with time. The coating of microporous filters with aluminum hydroxide flocs enhanced viral adsorption but caused clogging of the filters, therefore proving impractical for water filtration (Seeley and Primrose, 1979). Previous researchers showed that

forming ferric hydroxide flocs in the presence of sand leads to the coating of the sand with the ferric hydroxide. However, for the coating to be efficient this procedure would have to be repeated 17 times or the sand and ferric hydroxides would have to be mixed for 24-36 hours (Edwards and Benjamin, 1989; Mills et al., 1994). This time consuming preparation step makes this modified sand financially impractical. This coating, also, has the tendency to slough off the sand with time. Sand modified by coating with iron oxide was reported to out perform plain sand as a medium for filtration. The increased electropositive nature of this modified sand is believed to be responsible for its improved filtration and capture efficiency (Stenkamp, 1992). However, the process that was used for coating the sand was lengthy and the coating were not continuous. Iron oxide coating of particulate materials was reported by Farrah and Preston (1991) but the coating had a lower efficiency when large volumes of water were used and did not work as well as the metal hydroxide coating (Farrah et al., 1991).

Metallic peroxides have also been used in the modification of filter media. Gerba et al. (1988) incorporated magnesium peroxide coated solids in filters to adsorb viruses from water. Farrah and Preston (1991) found that coating diatomaceous earth with magnesium peroxide increased viral adsorption approximately tenfold. Filters containing magnesium peroxide coated diatomaceous earth recovered more viruses from trickling filter effluents than filters containing unmodified diatomaceous earth. In addition, filters containing magnesium peroxide modified diatomaceous earth inactivated bacteria that

usually colonize the filter media and prevented biofilm formation onto filter surfaces (personal observations).

In a method described by Farrah and Preston (1985), solids (sand, diatomaceous earth, micro porous filters) can be modified by In situ precipitation of metallic hydroxides directly onto their surface. Farrah et al. (1991) found that modified diatomaceous earth packed into filter holders adsorbed viruses very efficiently from water samples over a pH range of 4-9. Mansoor and Chaudhuri (1996) reported on the use of sand coated by a variety of the above methods in water filtration units in developing countries. Their study concluded that the metallic precipitate coating increased the efficiency of the filtration units. Also, studies on the removal of viruses, bacteria and protozoan parasites by columns or filters containing solids coated with metallic hydroxides have been reported (Lukasik et al., 1996; Lukasik 1997; Truisdail et al., 1998). In these studies the modified sand or diatomaceous earth was used in flow-through systems that was able to process large volumes of water and efficiently remove microbes for extended periods of time at different pH and temperature values.

Need for Improving Virus Capture and Detection.

Current methods for viral detection in environmental aqueous samples are not very sensitive. The two most commonly used and EPA approved methods for virus detection involve passing large volumes of water through filters under certain conditions. In one method the pH and ionic strength of the

water have to be adjusted prior to its passage through the 0.45 micrometer porosity Filterite filters. This method, which is time consuming, labor intensive and requires the passage of large volumes of water, is not very sensitive. The other method involves the passage of water through 1 MDS filters (Gerba, 1984; Gerba et al., 1988). Even though this method is easier, the filters are expensive and the viral adsorption and recovery efficiencies vary. Preliminary experiments and literature reviews show that 1 MDS filters did not perform as efficiently as claimed by the manufacturer in the removal of viruses. The concept used in these methods relies on maximizing attractive forces between viruses and the filtering material. In the 1 MDS filters, the viruses are attracted to the electropositive filter media. In the Filtrite filters, aqueous conditions are made suitable for virus adsorption to the filter fibers by lowering the pH to below the isoelectric point of most viruses to maximize electrostatic attraction (Shields et al., 1986). The relatively fair performance accompanied by the expensiveness and inconvenience of these methods emphasizes the need for finding efficient and cost effective methods that allow accurate and continuous viral monitoring of water samples.

Potential Uses of Improved Filter Media

If solids were to be modified so that it improves their filtration efficiency, then these solids could be very beneficial in three main areas. As stated earlier, the modified solids would be very beneficial under the following conditions:

1. If they could be used in drinking water treatment and/or water reclamation procedures, they would play a major role in enhancing both the biological and chemical qualities of the effluent and reduce the need for further disinfection. Many uses become apparent when considering the amount of reclaimed water used daily for industry, recreation, and source recharging. The state of California alone uses more than a million cubic meters a day of reclaimed waste water (Bitton, 1994).

2. If large point-of-use filters made with modified materials could be installed in rural areas or in developing countries where water quality is poor and a cause of public concern, they would be very beneficial in crisis times when biological water contamination becomes a concern and water consumption becomes hazardous.

3. If they could be used or implemented in compact point of use units that provide a means of removing contaminants from water at the source, such units would produce safer water without sacrificing the convenience and health of the users at a fraction of the cost of the more elaborate units.

4. If they could be used to make filters for concentrating viruses from water, this would be especially important when used for the detection and quantification of viruses since the current methods may not be so efficient.

Purpose of This Study

Due to the vast and essential role water plays in our everyday life, it is necessary for that water to be contaminant free; this includes both chemical and biological contaminants. As discussed previously, water had been implicated in the transmission of illness and is a reservoir for organisms that harbor or transmit disease. There is a wide variety of water sources for domestic use. These include ground water, surface water, desalinated water, and reclaimed water. The incidence of waterborne disease from these waters was related to the source and the level and extent of treatment of these waters. These occurrences depend on the general level of public health, the socioeconomic level of the people, geological and climatic conditions, sewage disposal practices, the technological development and degree of industrialization, and the degree of public concern and awareness. The incidence of waterborne diseases in the United States is low compared to the rest of the world; however, these outbreaks still occur and are responsible for the loss of billions of dollars and numerous lives every year. The issue of enhancing water quality is very important in undeveloped, developing, and developed countries. Improving current filtration practices and media could greatly improve water quality. Sand and diatomaceous earth are very commonly used in water filtration and are essentially renewable, reasonably efficient, readily available, and cheap. Thus, these materials were chosen in this study. By modifying these materials to better remove water contaminants, a flow through system capable of handling

large volumes of water can be obtained. Modification by the in situ precipitation of metallic hydroxides was chosen for coating these solids. Metallic hydroxides have been used for many years in the water treatment industry so little public health concern was associated with the safety of their use. The modification of sand and diatomaceous earth was performed by alterations of a method previously described by Farrah et al. (1988).

This dissertation summarizes the results of different modification techniques and their effects on the adsorption of microorganisms and other problematic water contaminants. Aluminum and ferric hydroxy/oxides coatings were used in this study. Coated solids were placed in different filtration apparatuses and subjected to daily use. Their performance was determined as a function of time and volume of water filtered. The removal of a variety of microbial and chemical contaminants was studied. Determining the biological and chemical filtration efficiency, the stability of the coating, and its lifetime were my main objectives. In addition, I studied the properties of the modified particles and the effects of salt solutions on the adsorption of viruses to coated or uncoated solids and microporous filters. This was performed in order to better understand the mechanisms involved in the adsorption of viruses to solids. The results of this study will aid in understanding the interactions between viruses and solids in aqueous environments and enable us to produce more efficient water filtration practices, and ultimately safer cleaner water.

Table 1. Major Infectious agents present in inadequately treated drinking water worldwide

Bacteria	**Viruses**	**Protozoa**
Capylobacter jujuni	Adenovirus (40/41)	*Balantidium coli*
Enteropathogenic E. coli	Enteroviruses (71 types)	*Giardia lambia*
Salmonella (1700 spp.)	Hepatitis A	*Cryptosporidium parvum*
Shigella (4 spp.)	Norwalk Agent	*Entamoeba histolytica*
Vibrio cholerae	Reoviruses	
Yersinia enterocolitica	Rotaviruses	
Helminths	Coxsackie Virus	
Ancylostoma duodenale		
Ascaris lumbricoides		
Echinococcus granulosis		
Enterobius vermicularis		
Necator americanus		
Strongyloides stercaralis		
Taenia (spp.)		
Trichuris trichiura		

Source: Data were adapted from Geldreich (1997).

CHAPTER 2
MODIFICATION AND CHARACTERIZATION OF THE SOLIDS

Previously, Farrah and Preston (1985) reported that solids (sand, diatomaceous earth, micro porous filters) can be modified by the In situ precipitation of metallic hydroxides directly onto their surface. According to this procedure, the solids are soaked in the metallic salt solution and allowed to dry. The solids are then soaked in a strong base solution, and the precipitates of the corresponding metallic hydroxides are formed onto their surface. Studies on the removal of viruses, bacteria and protozoan parasites in batch experiments or by columns or filters containing solids coated with metallic hydroxides have been reported (Cheng 1994; Lukasik et al., 1996; Lukasik, 1997; Truisdail et al., 1998). In these studies, coated sand or diatomaceous earth efficiently removed microorganisms from water at different pH and temperature values. The coated solids removed several orders of magnitude more microorganisms than uncoated solids. Their efficiency varied depending on the microorganisms, the size of the coated solids, the type and flow rate of filter system used, and the quality of the water containing the microbes. Since metallic hydroxides have been used for many years in water treatment processes (Bitton 1994), there is little concern about the safety of their use for coating filter media.

The aforementioned coating process is fairly crude and requires at least 48 hours to perform. The quality of the final coated material (even though superior to other mentioned coatings) varies from batch to batch. Previously, Lukasik (1997) reported that solids coated with a combination of ferric and aluminum chloride had the highest bacteriophage removal efficiencies. This combination removed greater than 99% of the challenge bacteriophages from water. Pursuant to that work, a similar combination was chosen to coat the solids used in this work.

In order to optimize the coating and reduce the batch-to-batch variability, it was necessary to apply the coating in a controlled manner that is both time and cost effective. A variety of coating techniques are studied and compared in the this chapter. In addition, I present the results I obtained on the physical and chemical characterization of the modified surfaces. The coating technique that resulted in solids exhibiting superior adsorptive character, stability, resistance to attritioning, and having the least batch to batch variation was chosen. Subsequently, this technique was used to coat large amounts of solids for use in long term, large scale filtration experiments described in the following chapters.

Physical and chemical characterizations of the surfaces of the modified and unmodified particles help gain an understanding of the nature of the adsorptive process. This characterization involves the study of the chemical composition of the surface, the shape of the surface, the overall charge of the particles, the hydrophobicity of the surface, the surface area, etc.

Determining the surface charge of the coated particles is essential for predicting resulting filtration efficiency of these particles. The charge associated with the surface of the molecule or solid contributes much to the adsorptive process. The effective charge of a particle is referred to as the Zeta Potential. The chemical makeup of the particle surface contributes greatly to the net charge the particle exhibits, and therefore the mechanism by which particles interact with that surface. If the coated solids are to be used commercially or industrially, they would have to retain their effectiveness for extended periods of time. Therefore, determining the extent of the attritioning and the amount of metals leaching of the coated solids is very important for assessing the usefulness of the modified particles in filtration processes. This involves the testing of the effluents of filters containing these modified material and determining the amount of metal coming off the filters . Also, the amount of deposited metal on the surface of the solids before and after their extended use must be determined.

In this chapter, I compare the various coating techniques and characterize the resulting coated solids. I examine the surface of the coated solids by various methods and measure factors dictating viral adsorption to coated or uncoated sand or diatomaceous earth. In addition, I will examine the effects of prolonged use on the metal content of the coated solids.

Materials and Methods

Modification of Sand and Diatomaceous Earth

Diatomaceous earth (grade 1) was obtained from Sigma Chemical Co., St. Louis, MO. Sand was obtained from local sources under the label "General Purpose Sand" and sieved to 35, 60 or 100 mesh. Dyna Sand was used in certain experiments and was obtained from the Parkson Filtration Company, Ft. Lauderdale, FL. Five different coating techniques were compared. The first coating method (method 1) was performed as described by Farrah et al. (1988). Briefly, the sand or diatomaceous earth was mixed with an equal volume of a solution containing 0.25 M ferric chloride and 0.5 M aluminum chloride for 30 minutes. Excess liquid was drained and the solids were allowed to air dry (24-48 hrs at room temperature). The samples were then mixed with approximately 2 volumes of 3 N ammonium hydroxide for 10 minutes and were allowed to dry (24-48 hrs). They were then rinsed with deionized water to remove excess precipitates. Coating method 2 used the same process as method 1 except for the difference in the drying steps. The 2 drying steps in this method were conducted in a oven dryer at 85°C. The solids were also stirred occasionally during drying to ensure even drying. This reduced each drying time to under 18 hours. Method 3 for coating the solids consisted of 2 sequential coating steps. First the solids were coated with ferric hydroxide, and then with aluminum hydroxide. This method was performed according to a method described by

Lukasik (1997). Briefly, the sequential coating of particles was accomplished by mixing the solids with 0.25 M ferric chloride. The excess liquid was drained and the solids were allowed to dry at 80°C (<24 hrs). The samples were then mixed with approximately 2 volumes of 3 N ammonium hydroxide for 10 minutes. They were allowed to dry at 80°C (<24 hrs). Then, they were rinsed with deionized water to remove excess precipitates. The same procedure was repeated again on the ferric hydroxide coated solids but by using 0.5 M aluminum chloride instead of the ferric chloride. Coating method 4 was performed as follows: the solids were placed in a container that was positioned on a vibrating platform. A 200 watt infrared lamp was placed directly above the container. The solids were vigorously agitated as they were being heated. When the temperature of the solids achieved about 85°C a mist of a solution consisting of 0.25M ferric chloride and 0.5M aluminum chloride in a 50% ethanol solution was sprayed onto the vibrating solids in short bursts. The spraying process was performed in 10 consecutive 3 second bursts with a 1 minute interval between sprays. The spray solution was warmed to about 60°C in a water bath. After the sprays were performed the solids were heated for 4 minutes and then a 60°C solution of 3M ammonium hydroxide was sprayed onto the solids in the manner mentioned above. The heating and vibration was maintained throughout the whole process. The modified solids were then rinsed and stored at room temperature until used. Figure 1 shows a schematic of the designed coating apparatus and vibrating chamber. Coating method 5, essentially utilized the same concept as method 4 but instead of heating with a infrared lamp a hot air blower was used.

Metal Content of Modified Particles and Filter Effluents

The metal deposited on the particles was solubilized by mixing samples of sand or diatomaceous with 5 volumes of 6 N HCl and agitating overnight at 37° C. The solids were separated from the solution by settling (sand) or by centrifugation at 300 g for 5 min (diatomaceous earth). The composition and amount of metal in the solutions were analyzed using an Inductively Coupled Argon Plasma (ICAP) spectrophotometer. Direct measurements of the metal content of Influent and effluent water samples from columns and filters containing modified and unmodified materials was done. Briefly, samples of filter or column effluents were taken at different time points in the course of the 30-90 day experiments. Also, samples of the coated sand were taken out of the filters or columns at different times. The coated sand samples were digested and analyzed for metal content as described.

Coating Durability Tests

Triplicate 5g samples of sand coated by different methods were placed in conical centrifuge tubes. Twenty five milliliters of water was added to each tube. The tubes were placed horizontally on a rocking shaker and were agitated at the highest setting for 48 hours. The liquid was then decanted, the sands were rinsed with deionized water and dried. The sand were then digested in two volumes of 5 N hydrochloric acid overnight as previously mentioned. The digestate was then analyzed by ICAP.

Zeta Potential Measurements

The zeta potential of the coated and uncoated sand was measured by Steve Truisdale of the Department of Chemical Engineering at U.F. by a streaming potential apparatus. The zeta potential of the modified and unmodified diatomaceous earth particles was measured in 0.02 M glycine 0.02 M immidazole buffer at pH 7.3 using a Lazer Zee model 501 zeta meter.

Scanning Electron Microscopy

Scanning electron microscopic examination of the modified solid materials was performed using a Hitachi scanning electron microscope at the department of Zoology at the University of Florida. Surface elemental analyses for the mapping of aluminum and iron species on the surface of the sand was performed by an elemental microprobe X-ray analyzer equipped SEM in the Department of Material's Science and Engineering.

Statistical Analysis

Standard deviations, correlations, and general t test probabilities were determined using PSI-Plot software (Poly Software International, Salt Lake City, Utah).

Results

Metal Content of Metal Hydroxide Coated Solids

The amount of aluminum and ferric deposited on the different solids by different methods is shown in Table 2. All rinsed batches of sand that were coated by methods 2, 3, 4, and 5 contained slightly more iron and aluminum than sand coated by method 1. Generally, 35 mesh sand contained on the average 1.6 mg and 3.6 mg of iron and aluminum, respectively, per gram of sand. One hundred mesh sand contained 8.3 mg and 13.5 mg of iron and aluminum, respectively, per gram of sand. Diatomaceous earth coated by method 2 contained 78 mg and 108 mg of iron and aluminum, respectively, per gram of diatomaceous earth analyzed. Analyzing the metal content of coated sand after its prolonged use in a commercial swimming pool filter or in filtration columns gave comparable metal content results. Neither iron nor aluminum species were detected in column and filter effluents. Even when the water was re-circulated through the filter or column repeatedly for a 1 week period in a closed loop fashion, no metals were detected in the effluent water. The limits of detection of the ICAP instrument used were 0.01 mg/l for both iron and aluminum.

Coating Durability Tests

Sand coated according to methods 2, 3, 4, and 5 had substantially more metal on its surface after vigorous shaking than sand coated according to method 1 (Table 3). Sand coated according to method 1 lost about 60% of its

original coating. However sand coated with the other mentioned methods only lost on the average of 30% of its original coating.

Zeta Potential

The zeta potential of the modified 100 mesh sand was between 20 and 30 mV regardless of the method used to coat the sand (Table 4) The zeta potential of the diatomaceous earth modified with the different coating methods averaged about +10 mV. The zeta potential varied slightly for the solids when different coating methods were employed. However when a t test comparison was performed the zeta potentials of the coated solids were not statistically different. Unmodified diatomaceous earth had an average zeta potential of -68mV at pH 7.4. Unmodified sand had a zeta potential of -93mV at pH 7.4.

Scanning Electron Microscopy

Scanning electron microscopic examination of coated sand by the different methods revealed a layer of coating on the surface of the sand (Figure 2). Surface examination of sand coated according to method 2 revealed a more continuous coating than that generated on the surface of the sand by method 1. Examining the surface of the coated sand with the elemental microprobe X-ray analyzer revealed the abundance of iron and aluminum species on the surface of the sand. Metal intensities and densities on the surface of the sand were higher for the sand coated by methods 2, 3, 4, and 5 than those for method 1 (Figure 3).

Discussion

Charged particles are attracted to particles and surfaces that possess an opposite charge. Inorganic and organic materials, including microorganisms have a charge associated with their surface and follow the same principle. Sand, diatomaceous earth and most biological particles poses an overall electronegative charge at normal environmental water pH values. Based upon this, the colloid interaction theory also known as the DVLO theory explains their adsorption on the bases of double layer interactions. If the sand or diatomaceous earth could be coated and made less negative, then biological particles are more likely to adsorb onto their surface via electrostatic attractions.

The amount of iron and aluminum hydroxide deposited on the coated sand and diatomaceous earth may dictate the effectiveness of the coating. The different described methods coated the solids with a substantial amount of metal. Slight differences existed between the amount of metal initially deposited by the different methods. In general diatomaceous earth contained about 20% metal (iron and aluminum) by weigh, 100 mesh sand contained about 2% metal by weight, and 35 mesh sand contained about 0.5% metal by weight. Even though the amount of deposited metal was the same by the different coating techniques, the durability of the coating differed. Solid coated according to method 2, 3, 4, and 5 displayed higher resistance to attritioning. These solids contained substantially more metal after shaking for extended periods of time.

The zeta potential of the coated sand or diatomaceous earth was relatively the same when different methods were used to coat the solids. After attritioning, the zeta potential of solids coated according to method 1 decreased more than those of the solids coated according to method 2,3,4, and 5 (data not shown). The decreased electronegativity of the coated particles dictated their effectiveness in removing microorganisms from water. Lukasik (1997) reported that the zeta potential of the modified solids is directly related to the adsorption of both bacteria and bacteriophages. Efficient adsorption of these microorganisms occurs once the zeta potential is raised above approximately -30 mV (for phages) and - 10 mV (for bacteria).

From the above observations it was concluded that methods 2,3,4, and 5 produced a coating that was more durable and superior than the original coating Method # 1. The solids produced by methods 2-5 were equally efficient in removing viruses from water. Coating method 3 produced a slightly more efficient coating, however this method was the most time consuming and therefore was abandoned. Method 4 and 5 produced solids that were just as effective as method 2 and the whole coating procedure was performed in under 30 minutes. However, only small batches of solids can be coated at a time due to the size of the designed agitation chamber. Therefore, methods 4 and 5 were not chosen to further coat solids for the large scale filtration experiments. This was due to the fact that resources for the scale up of the apparatus shown in Figure 1 were not available. Method 2 was chosen for coating solids that will be used in large filtration columns or sand bed filters or faucet filters.

Neither iron nor aluminum were detected in the effluents of filters containing coated sand. However, if leaching would occur, the metals released are very unlikely to produce toxic byproducts. Attritioning of the coating was also not observed after prolonged use since the modified solids contained the same amount of metal before and after prolonged use. Therefore, attritioning and leaching do not seem to limit the length of time that the filters could be used.

Electron micrographs of the modified solids (method 2) show a smooth and continuous surface. The coating appears to be deposited evenly throughout the surface of the particles. Thus, this process aids in smoothing out surface irregularities. Elemental microprobe SEM analysis of the surface reveals the even distribution of the ferric and aluminum on the surface. Coating methods 2-5 show higher densities of aluminum and iron on the surface of the coated sand.

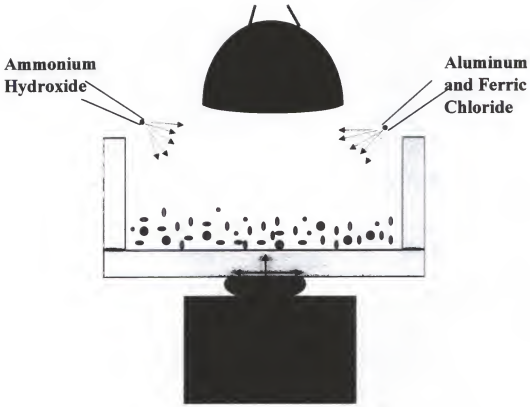

Figure 1. Schematic of the developed particle coater used in method 4.

Note: A similar set up was used in method 5 but a hot air blower replaced the infra red lamp.

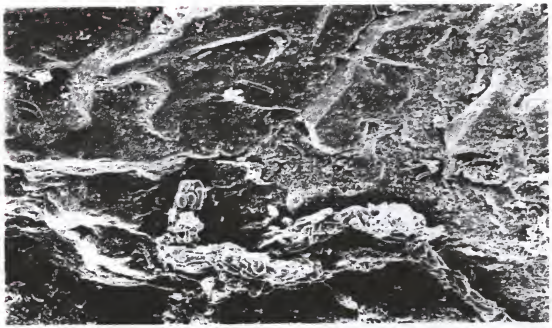

Figure 2. Scanning electron micrograph of the surface of sand coated according to method 2.

Note: the coated sand was briefly exposed to wastewater.

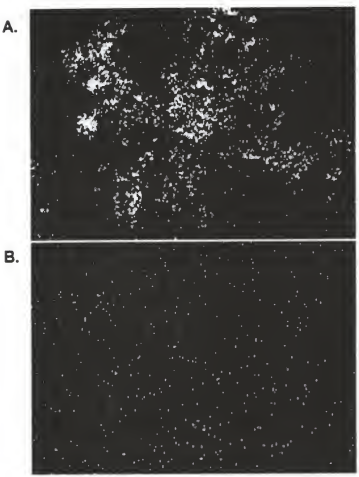

Fig. 3 Electron microscope XPS elemental analysis of sand coated with ferric and aluminum hydroxides A. aluminum, B. Iron

Table 2. Amount of iron and aluminum deposited on the coated sand as determined by ICAP of the digestate.

Coating Method	PPM metal on the Surface of Coated Solids					
	35 Mesh Sand		100 Mesh Sand		Diatomaceous Earth	
	Iron	Aluminum	Iron	Aluminum	Iron	Aluminum
Method 1	1.2±0.2	3.2 ±0.3	8.0±0.4	13.3±1.1	68±10	111±22
Method 2	1.5±0.1	3.7 ±0.2	8.4±0.2	13.6±0.5	79±5	106±7
Method 3	1.4±0.2	3.4 ±0.2	8.5±0.3	13.4±0.4	81±4	108±9
Method 4	1.6±0.1	3.7 ±0.2	8.4±0.2	13.7±0.3	82±6	104±5
Method 5	1.5±0.1	3.6 ±0.1	8.2±0.1	13.3±0.3	Not performed	

Note: Five grams of sand or 1 gram diatomaceous earth were digested in 25 ml of 6N HCl at 37°C over night then it was analyzed by ICAP. Uncoated sand contained less than 0.2 ppm iron and aluminum at all times.

Table 3. The effect of attritioning the metal content of 35 mesh sand coated by the different methods.

Coating Method	Amount of metal on the sand after attritioning	
	Iron	Aluminum
Method 1	0.4±0.05	1.4±0.2
Method 2	1.1±0.1	2.6±0.3
Method 3	1.2±0.2	2.7±0.4
Method 4	1.2±0.1	2.7±0.3
Method 5	1.1±0.1	2.6±0.3

Note: coated 35 mesh sand was shaken vigorously in water for 48 hrs. and then digested in 6N HCl acid overnight. The digestate was then analyzed by ICAP.

Table 4. The Zeta potential of 35 mesh sand or diatomaceous earth (DE) coated according to the different methods.

Modified solids and Coating Method	Zeta Potential
15 mesh sand/ Method 1	+20±5
15 mesh sand/ Method 2	+25±10
15 mesh sand/ Method 3	+28±10
15 mesh sand/ Method 4	+25±10
Uncoated 15 mesh sand	-90±15
DE/ Method 1	+8±5
DE/ Method 2	+11±4
DE/ Method 3	+10±5
DE/ Method 4	+13±3
Uncoated DE	-68±5

Note: The Zeta potential of 15 mesh sand was measured by a streaming potential apparatus. Diatomaceous earth zeta potential was measured by the Lazer Zee apparatus.

CHAPTER 3
REMOVAL OF VIRUSES FROM WATER BY COLUMNS OR SWIMMING POOL FILTERS CONTAINING MODIFIED PARTICLES

The presence of microbial species in our water compromises the quality of the water and jeopardizes our health. Many current large scale water filtration or treatment practices are unable to remove viruses and other microorganisms. The most problematic of these microorganisms has been *Cryptosporadium parvum*. These oocysts have been detected in water treatment plant effluents and have caused numerous disease outbreaks. As recently as July of 1998, *Cryptosporadium parvum* oocysts tainted the drinking water supplies of Sydney, Australia. Although water treatment processes were operating at optimum levels, the outbreak still occurred. Almost three and a half million people were advised to boil all drinking water by the Australian government. This advisory lasted for 30 days. The inherent high chlorine resistance of these pathogens renders their control problematic. Many water plants are thus forced to employ extensive filtration practices. This again reiterates the need of filtration media capable of the efficient removal of pathogens.

In the previous chapters we discussed the prevalence of viral and protozoan pathogens in drinking and wastewater, and their association with

water borne disease outbreaks. Microorganisms have also tainted recreational waters. Cases of disease transmission associated with bathing in recreational waters have been reported (D'Alessio et al., 1981; Hawley et al.,1973; Kee et al., 1994; Lenway et al., 1989; MMWR, 1997; Turner et al., 1987). As with waste and drinking water, the responsible etiological agents could be viral, bacterial or protozoan. Most of the resulting symptoms of infection include: diarrhea, nausea, vomiting and abdominal pain. More than likely, many cases of milder infections go unnoticed and/or unreported. Disinfection and filtration are common methods used to control pathogens in swimming pool water. Diatomaceous earth and sand are commonly used filter media in swimming pool filters. Diatomaceous earth filters have the advantage of the enhanced removal of microorganisms (Brown et al. 1974), but are subject to frequent clogging, flow restriction, and their use can often raise health concerns. These health concerns are related to the possible role of diatomaceous earth particles in lung diseases. Sand filters have been extensively used in water filtration processes (Bitton, 1994). These filters remove relatively small numbers of pathogenic microorganism from water. Their performance can be improved by allowing the development of a biofilm on the sand surface. Also, the microbe removal efficiency can be increased by the addition of coagulants (polymeric compounds or metallic hydroxides). Although useful for water and waste water treatment, these procedures are not practical for swimming pools. Swimmers may object to the presence of coagulants and long ripening periods for filters are not possible. Filtration of swimming pools is usually combined with disinfection. Chlorine is

the most common disinfectant used in water treatment. It is effective in killing a wide range of microorganisms. However, its use is limited to low concentrations since it is caustic and may produce toxic byproducts by reacting with organic materials.

The inherent inability of sand and diatomaceous earth to adsorb microorganisms from water prompted the search for methods that could modify these solids and increase their adsorptive character. The modified particles would have to be environmentally friendly by not releasing any harmful chemicals, and operate over a broad pH and temperature range. They also would have to be stable for relatively long periods of time, and efficiently adsorb microorganisms from various waters. It has been a long known fact that ferric hydroxide and aluminum hydroxide flocs are useful in treating water and waste water (Bitton 1994). These flocs adsorb microorganisms through hydrophobic and electrostatic interactions, and remove them from water following settling or filtration. The ability of metallic hydroxides to remove microorganisms and other pollutants has led to attempts of coating solids with them. Previously, researchers have shown that solids coated by the in-situ precipitation of metallic hydroxides may possess some of these desirable characteristics (Mansoor Ahmed and Chaudhuri, 1996; Edwards and Benjamin, 1989; Farrah et all., 1991; Lukasik 1997; Lukasik et al., 1996). The low production cost and effectiveness of these coating renders them attractive for potential use in large scale water filtration processes. The modification of solids with hydroxides of metallic salts

raises their Zeta potential, therefore, decreasing their electronegativity. This results in the strengthening of the electrostatic interactions between these solids and the suspended microorganisms over a wide pH range (3.5-9.5). By incorporating the modified solid materials into filtration units, filtration and adsorption are combined to result in enhanced removal efficiencies. These filters would be useful for emergency use and as advanced filtration units in developed countries or as low cost point of use water treatment systems in developing countries.

Previously, Lukasik (1997) showed that sand or diatomaceous earth coated with ferric and aluminum hydroxide removed more microorganisms than sand coated with ferric hydroxide, aluminum hydroxide, or magnesium hydroxide. Also shown, was that metallic hydroxides out performed metallic oxides in removing viruses from water in batch experiments. In the previous chapter, solids coated with ferric and aluminum hydroxides were tested for the durability of their coating. In this chapter, these solids were first compared for their removal of bacteriophages and polio1 from water when placed in small columns. The method that generated solids with the most desirable performance was chosen for coating large amounts of solids for further, more in depth studies. Solids coated according to this method were placed in large columns, swimming pool filter units, or faucet water filters. Uncoated sand was used in these units in order to provide a negative control. Water (or simulated sewage) was passed through these columns or filters daily and they were occasionally challenged

regularly with viral, bacterial and protozoan pathogens. Percent removals of the different pathogens by these units were determined.

In order to evaluate the performance of the filtration units, already established procedures for detecting the seeded pathogens, and newly developed procedures were used. The efficiency of the different procedures for determining the presence and viability of different viral pathogens in the water samples was compared. This allowed the evaluation of novel methodologies for the detection of viral and protozoan parasites from water samples. The results obtained from this evaluation will aid in the development of more efficient procedures for the detection of pathogens from environmental water samples.

In summery, in this chapter I define a coating technique that generates solids that demonstrating superior pathogen removals from water. I also evaluate the different methodologies for detecting the presence and viability of various viral and protozoan pathogens in water samples.

Materials and Methods

Modification of Sand

Sand was obtained from local sources under the label "General Purpose Sand" and sieved to 35, 60 or 100 mesh. Dyna Sand was used in certain experiment and was obtained from the Parkson Filtration Company, Ft. Lauderdale, FL. Four different coating techniques were compared. The first coating method (method 1) was performed as described by Lukasik et al. (1996).

Briefly, the sand or diatomaceous earth was mixed with a equal volume of a solutions consisting of 0.25 M ferric chloride and 0.5 M aluminum chloride for 30 minutes. Excess liquid was drained and the solids were allowed to air dry (24-48 hrs). The samples were then mixed with approximately 2 volumes of 3 N ammonium hydroxide for 10 min and were allowed to dry (24-48 hrs). They were then rinsed with deionized water to remove the excess precipitates. Coating method 2 used the same process as method 1 except for the difference in the drying steps. The 2 drying steps in this method were conducted in a oven dryer at 85°C. The solids were also stirred up occasionally during drying to ensure thorough heating and drying. This reduced each drying time to under 18 hours. Coating method 3 consisted of 2 sequential coating steps; first the solids were coated with ferric hydroxide and then aluminum hydroxide. This method was performed according to a method described by Lukasik (1997). Briefly, the sequential coating of particles was accomplished by mixing the solids with 0.25 M ferric chloride. The excess liquid was drained and the solids were allowed to dry at 80°C (<24 hrs). The samples were then mixed with approximately 2 volumes of 3 N ammonium hydroxide for 10 min and were allowed to dry at 80°C (<24 hrs). They were then rinsed with deionized water to remove excess precipitates. The same procedure was repeated again on the ferric hydroxide coated solids but by using 0.5 M aluminum chloride instead of the ferric chloride. Coating method 4 was performed as follows: the solids were placed in a container that was positioned on a vibrating platform. A 200 watt infrared lamp was placed directly above the container. The solids were vigorously agitated as

they were being heated. When the temperature of the solids achieved about 85°C, a mist of a solution consisting of 0.25M ferric chloride and 0.5M aluminum chloride in a 50% ethanol solution was spayed onto the vibrating solids in short bursts. The spraying process was performed in 10 consecutive 3 second bursts with a 1 min interval between sprays. The spray solution was prewarmed to about 60°C in a water bath. After the sprays were performed the solids were heated for 4 min and then a 60°C solution of 3M ammonium hydroxide sprayed onto the solids in a the manner mentioned above. The heating and vibration was maintained throughout the whole process. The modified solids were the rinsed with deionized water and stored at room temperature until used. Figure 1 shows a schematic of the designed coating chamber. Coating Method 5, essentially utilized the same concept and apparatus as method 4, but instead of heating with a infrared lamp, a hot air blower was used to dry the sprayed solids.

Bacteria

The following bacterial strains were used in removal studies: *Escherichia coli* C-3000 (ATCC 15597), which was routinely grown in Tryptic Soy Broth (TSB) and assayed using MacConkey Agar (Difco Labs, Detroit, MI); and *Staphylococcus aureus* (ATCC 12600), which was grown on TSB and assayed on Manitol Salt Agar (Difco Labs, Detroit, MI).

Detection Bacteriophages and Enteric Viruses by Standard methods

Polio virus 1 (strain Lsc), Echo virus 1 and 3, and Coxsackie B 3 and 5 virus were grown on buffalo green monkey (BGM) or RD cells and assayed as

plaque forming units (PFUs) by using an agar overlay technique (Smith and Gerba, 1982). Rotavirus SA11 was propagated on MA104 Cells and assayed in a method comparable to the one previously described by Smith and Gerba (1982). Briefly, The sample was allowed to incubate on the MA104 monolayer for 45 min. Serum free minimum essential medium with L-Glutamine, antibiotics, and 0.0005% trypsin was added and the flasks were incubate at 36 degrees for one week. The cells were checked for Cytopathic effects daily. Flasks demonstrating positive cytopathic effects were verified to contain rotaviruses using a Rotazyme EIA kit (Abbot Diagnostics). The Most probable number of viruses was calculated using software supplied by the Environmental protection agency (1994). The following bacteriophages and their host bacteria were used: MS2 (*Escherichia coli* C-3000, ATCC 15597); ϕX 174 *(Escherichia coli,* ATCC 13607); PRD-1 (*Salmonella typhimurium*, ATCC 19585). Bacteriophages were grown on their respective hosts and assayed by soft agar overlay technique (Snustad and Dean, 1971).

Detection of Enteroviruses by RT-PCR

The detection of enteroviral specific nucleic acids sequences was achieved by the enzymatic amplification of the cDNA copy of the viral RNA. The implemented procedure was based on a single tube reverse transcriptase-polymerase chain reaction (RT-PCR) amplification procedure. RT of viral genomic RNA was carried out as previously described (Shieh et al. 1997), except sequence specific primers were used instead of random hexamers, and

Tfl polymerase was used instead of the Taq polymerase. The primers used were specific to sequences in the highly conserved 5' non translated region. Table 5 gives the sequences of the primers used for the detection and amplification of enteroviral RNA. Briefly, 20 µl sample was incubated at 99° C for five minutes to release the viral RNA from the capsids. Subsequently, 10 µl of that sample, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, 200 µM of each dNTPs, 2 units AMV reverse transcriptase, 2 units Tfl DNA polymerase, 0.5 µM of forward primer, 0.5 µM reverse primer, and 10 units RNAsin (RNase inhibitor) were placed into thin walled PCR tubes placed on ice. The final volume in each tube was 50 µl. All the above reagents were obtained from Promega, Madison, WI. All primers were synthesized by Genosys Biotechnologies, Woodlands, TX. The tubes were then transferred to a Perkin Elmer 9600 thermal cycler. The following profile was run: 46° C for 45 min (RT), 99° C for 5 min. (denature AMV rt), 35 PCR cycles of (95°C for 90 sec./ 55°C for 60 sec./ 72°C for 90 sec) and a final extension step of 72° C incubation for 10 min. Rt-PCR products were then electrophoresed on a 2% agarose gel containing 0.001% Gelstar (FMC Bioproducts, Rockland, ME). The DNA bands were visualized under UV lighting. Negative controls containing DEPC treated sterilized double deionized water were run at all times. The Most probable number of viruses was calculated using software supplied by the Environmental protection agency (1994). To allow for the use of larger sample volumes, the RNA in the original virus sample was concentrated by the use of a QIAGEN RNeasy Kit (Santa Clarita CA). Much care was taken to reduce the amount of RNase contamination in the work

stations. Gloves were worn at all times, all prepared solutions were DEPC treated, and lab tops and pipetters were cleaned regularly with RNase away (Fisher Scientific, Pittsburgh, PA).

Viable Enteroviruses Detection by Integrated Cell Culture RT-PCR

Integrated cell Culture RT-PCR (ICC/RT-PCR) was adapted for the use of the RT-PCR reaction to detect viable infectious enteroviruses. In this method water samples were first inoculated onto BGM or RD cells as described by Smith and Gerba (1982) and allowed to incubate for 48 hours. The flasks containing the cells were then frozen and rapidly thawed three times. The cell lysates were then centrifuged at 10 x g for 10 min. and the supernatants were subjected to RT-PCR as described above. The Most probable number of viruses was calculated as previously described.

Detection of Rotaviruses by RT-PCR

A similar approach was used to detect rotaviruses as the one used to detect the enteroviruses with exception that rotavirus specific primers were used (Table 5). In addition, seminested PCR was performed to assure the detection of rotavirus specific sequences. Basically, 20 µl sample was incubated at 99°C for 5 min to release the viral RNA from the capsids. Subsequently, 10 µl of that sample, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, 200 µM of each dNTPs, 2 units AMV reverse transcriptase, 2 units Tfl DNA polymerase, 0.5 µl of forward primer, 0.5 µl reverse primer, and 10 units RNAsin (RNase inhibitor) were placed into thin walled PCR tubes placed on ice. The final volume in each tube was 50

µl. All the above reagents were obtained from Promega (Madison, WI.) The tubes were then transferred to a Perkin Elmer 9600 thermal cycler. The following profile was run: 46° C for 45 min (RT), 99° C for 5 min (denature AMV rt), 35 PCR cycles of (95°C for 90 sec./ 55°C for 60 sec./ 72°C for 90 sec) and a final extension step of 72° C incubation for 10 min. Five microliters of this PCR product was used in a second PCR reaction that contained the internal primer instead of the reverse primer in addition to all the other ingredients. The second PCR was run according to the same profile. The final PCR products were then separated on a 2% agarose gel containing 0.001% Gelstar (FMC Bioproducts, Rockland ME). The DNA bands were visualized under UV lighting. Negative controls containing DEPC treated double deionized water were run at all times. Previously mentioned precautions for reducing RNase contamination were practiced. The most probable number of viruses was calculated as previously described.

Protozoan Parasites

Viable oocysts of *Cryptosporidium parvum* were obtained from Waterborne Inc. (New Orleans, LA). For enumeration, an immuno-flourescent assay (IFA) was used. For this purpose, the Cryptoglow staining kit (Waterborne Inc, New Orleans, LA) was used according to the manufacturers instructions and the oocysts were visualized and counted under an epifluorescence microscope.

Detection of *Cryptosporadium parvum* by PCR, RT-PCR, and Cell Culture Integrated PCR (ICC/RT-PCR)

The detection of *Cryptosporadium parvum* specific nucleic acids sequences was achieved by the enzymatic amplification of their DNA. A conserved sequence of the hsp 70 gene was amplified and detected as previously described (Rochelle et al., 1995). The nucleic acids were liberated from the oocysts by 8 cycles of liquid nitrogen freeze and boiling water thaw. The DNA was extracted by chloroform phenol procedure or by the use of a BioRad Prep A Gene DNA Extraction Kit (Hercules, CA). For the PCR, the sequence of the primers used is given in Table 5. The PCR was carried out as follows: 99° C for 5 min, 35 PCR cycles of (95 °C for 60 sec./ 55 °C for 30 sec./ 72 °C for 60 sec) and a final extension step of 72 C incubation for 10 min. each reaction contained 10 µl of lysed sample , 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, 200 µM of each dNTPs, 2 units Tfl DNA polymerase, 0.5 µl of forward primer, and 0.5 µl reverse primer. The final volume in each tube was 50 µl. All the above reagents were obtained from Promega, Madison, WI. All primers were synthesized by Genosys Biotechnologies, Woodlands, TX. The PCR products were then electrophoresed on 2% agarose gel containing 0.001% Gelstar (FMC Bioproducts, Rockland, ME). The DNA bands were visualized under UV lighting. Negative controls containing DEPC treated sterilized double deionized water were run at all times. For cell culture integrated PCR samples containing *Cryptosporadium parvum* were inoculated onto HCT-8 cell line. They were allowed to grow for 72 hours. The cell were then lysed and their DNA or

RNA was extracted. A PCR assay was performed as mentioned above on the DNA fraction. From the RNA fraction, the mRNA was isolated by the use of the Tri reagent or a mRNA Isolation Kit (Sigma, St Louis, MD) and an RT-PCR was performed using the same primers used for the PCR. An amplification profile identical to that of the viral RT-PCR was used and the bands were visualized in a similar manner. The most probable number of oocysts was calculated using software supplied by the Environmental protection agency (1994).

Removal of Microorganisms by Sand Coated by Various Methods

Thirty five mesh sand coated by the previously described methods was packed into separate small columns (24 X 2.5 cm). For each coated sand batch, three column were set up. Each column contained 40g of sand. Glasswool (Fisher Scientific, Pittsburgh, PA) was placed in the bottom of each columns. Before being used in experiments, deionized water was passed through the columns till the unbound metallic hydroxide was removed and the column effluents were clear and free of precipitates. One hundred and sixty milliliters of water buffered to pH 7 (0.02M glycine and 0.02M immidazole) and seeded with different bacteriophages was passed through the columns by gravity flow The columns effluents were collected and assayed for the seeded bacteriophages. Triplicate determinations of the number of viruses in the column's influents and effluents were performed, and the removal efficiencies were calculated. Identical experiments to the above were performed using sand that had been attritioned (as described in Chapter 2) prior to packing into the columns.

Large 100 Mesh Sand Column Setup and Viral Removal

One kilogram of sand coated according to method 2 or uncoated sand was placed in columns (35.0 X 5.0 cm). Glass wool (Fisher Scientific, Pittsburgh, PA) was placed at the bottom of each column to retain the sand. Two columns containing coated sand and two columns containing uncoated sand were set up. Before being used in experiments, deionized water was passed through the columns till the unbound metallic hydroxides were removed and the column effluents were clear and free of precipitates. Water was passed through the columns under a positive pressure by the use of pressurized nitrogen gas. The flow rate through the columns was approximately 450 ml/min. Four liters of tap water were dechlorinated by the addition of sodium thiosulfate (0.0002% final concentration) and adjusted to 0.02M glycine and 0.02M immidazole and pH 7. Bacteriophages, polio 1 and rota virus SA11 were added to the water to achieve a concentration of approximately 10^5 pfu/ml. The seeded water was forced through the column at a rate of 450 ml/min. After the passage of the first 1000 ml, triplicate samples of the column effluents were taken. Both the initial samples and the column effluents were assayed for the seeded microorganisms. Four liters of water was passed through the coated or uncoated sand columns at a rate of 450 ml/min daily for 48 days. Throughout this period, occasionally viruses were added to the water samples and were passed through the columns. At those times, both the initial samples and the column effluents were assayed for the these microorganisms. By the end of the

study, One hundred and ninety three liters of water had been passed through each of the sand columns.

35 Mesh Sand Column Filter Setup and Pathogen Removal

Uncoated 35 mesh sand was packed in polycarbonate columns that were 25 cm tall and had an interior diameter of 5 cm. Coated sand was also packed in the same manner in separate columns. Approximately, 500 ml (750g) of sand was placed in each column. Eighty mesh plastic screens were placed in the bottom of each columns. Water was passed downward through the columns by Little Giant pond pump (Little Giant, Okla City, OK) that was placed in a 40 liter tub. A schematic of this set up is shown in Figure 4. Twenty liters of deionized water was initially passed through the columns and discarded to rinse the loose debris of the sand. Different viruses were added to 25 liters of dechlorinated (pH 7-8) tap water to obtain a initial concentration of approximately 10^5 pfu/ml. In separate experiments oocysts of *Cryptosporadium parvum* were added to 25 liters of dechlorinated (pH 7-8) tap water to obtain a initial concentration of approximately 10^2 oocysts/ml. The water in the tub was recirculated by the pump for 5 min to disperse the microorganisms. Samples of that water were taken and the water was passed through the columns at a flow rate of 900 ml/ min. and a superficial velocity of 0.82 cm/sec. Five liters of the seeded water was passed through each column. Triplicate samples of inffluent and effluent were assayed and used to determine the percent removals of the different microorganisms. In separate experiments, 20 liters of dechlorinated water

were seeded with microorganisms and run through the column. The column's effluents were allowed to flow back into the tub containing the Initial 20 liters of seeded water. The water in the tank was recirculated through the columns for 3 hours. Triplicate Initial samples were taken at the beginning of the experiment. Samples of column effluents were taken at time zero and every 30 min for three hours. The concentration or presence of microorganisms in the initial and effluent samples at every time point was determined by various methods.

Swimming Pool Filter Set Up and Microorganism Removals

Two identical filtration systems were used; one contained sand coated according to method 2 and the other contained unmodified sand. Each filter system consisted of a Porpoise 180 commercial swimming pool filter (Porpoise, Jacksonville, FL) that was run by a 40 gal/min timer controlled electrical pump connected to 100 gallon water container. A schematic of the pool filter set up is presented in Figure 5. Each filter contained 220 Kg of sand in a 0.05 M^3 area. Tap water was circulated through the filter for 5 hours per day during the 170 day test period. A total chlorine residual of 2 ppm was maintained in the water tanks while the filters were running. The water in the tanks was adjusted to a residual of 10 ppm once a week to mimic super chlorination practices in swimming pool water treatment. Chlorination was performed by the addition of household bleach. Residual chlorine was determined using O-tolidine (American Public Health Association, 1989). The tank water was frequently changed, and samples were taken for metal analysis. Throughout the test period bacteria,

protozoan parasites, and various viruses were added to 50 gallons of dechlorinated tap water (pH≈7) to obtain a initial concentration of approximately 10^5 pfu or cfu or oocysts/ml. The tanks were mechanically agitated for 15 min and samples were taken before filtration. Fifty gallons of the seeded water was then passed through each filter and collected. Triplicate samples of inffluent and effluent samples were assayed and used to determine the percent removals of the different microorganisms. In separate experiments, 100 gal of water were seeded with microorganisms and the water was circulated through the filter for one hour. Triplicate samples were taken out before and after circulation and percent removal of the test microorganisms was determined. Before seeding with bacteria or viruses, the tanks were drained and filled with water that had been dechlorinated by the addition of sodium thiosulfate.

Tap Water Faucet Filters

Faucet filters were obtained from Ace hardware (model # 3857). Dr. Gary Hatch from AMETEK kindly provided empty filter cartridge shells that were to contain the modified solids. A total of six filter shells were used in this study. Each filter shell contained a bottom 2.75 g layer of coated (method 2) diatomaceous earth. Directly above that layer, 60g of coated (method 2) 100 mesh sand was added. Finally, 20g of activated carbon was placed on the top and the filter shell was sealed (Figure 6). The faucet filters containing the solids were attached to a faucet and 10 liters of tap water was run through these filter daily for 25 days. All faucet filter experiments were performed at room

temperature. However, three of the filters were stored at 4°C in between uses, and the other 3 filters were kept at room temperature. Initially, and once a week these filters were challenged with water containing different viruses. These experiments were performed as follows: various viruses were added to four liters of dechlorinated tap water (pH~8) to obtain a initial concentration of approximately 10^5 pfu/ml. The water was placed into a 4 liter pressure vessel connected to a compressed nitrogen tank at one end, and to the faucet filter at the other end. The water was forced through the filters and collected. The flow rate through the columns was about 1.5 ml/sec. The numbers of microorganisms in the filter effluents were determined and the corresponding percent removals were calculated. Experiments using filters containing uncoated sand and diatomaceous earth or activated carbon were also conducted and their percent removals of each of the tested viruses were determined.

Statistical Analysis

Standard deviations, correlations and general t test probabilities were determined using PSI-Plot software (Poly Software International, Salt Lake City, Utah).

Water Metal Analysis

Water samples from the swimming pool filter, sand column, and faucet filter effluents were taken at numerous occasions. The samples were analyzed by Inductive Coupled Argon Plasma Spectroscopy to determine the presence and concentration of metals in the water.

Results

Removal of Microorganisms by Sand Coated by Various Methods

The results of the removal of bacteriophages and polio1 by 35 mesh sand coated according to the various methods are shown in Table 6. Initially, the removal of the microorganisms by the different sands were fairly comparable. However after subjecting these sands to extensive attritioning, the removal of microorganisms by sand coated according to method 1 drastically declined. Sand coated according to the other methods did not experience a similar drastic decline in efficiency. These sands were still able to remove (on the average) greater than 98% of the challenge viruses. Overall, sand coated according to method 2, 3, 4, or 5 out performed sand coated according to method 1 both in long or short term use. Therefore coating method 2 was chosen for coating large quantities of sand for further experiments

Removal of Microorganisms by Large 100 Mesh Sand Columns

Large columns containing coated sand (method 2) reduced the number of MS-2, PRD1, and ϕX174 by more than 99.99% The remained greater than 99% even after the passage of 148 liters tap water (Figure 7). After 192 liters of water was passed through the columns, these columns still removed 99.9% of the MS-2 virus. The removal of PRD-1 was around 95% and the removal of ϕX174 dropped to around 90%. Columns containing modified sand reduced the

level of polio 1 by 4.1 $\log_{10}$ units at day 1. More than a 3 $\log_{10}$ reduction was obtained after the passage of 120 liters of water. After the passage of 192 liters either columns removed approximately 90% of polio 1 from tap water. Columns containing untreated sand removed less than 50% percent of the challenge viruses through out the passage of the first 148 liters of water (Figure 8). However this removal improved to around 90% towards the end of the test period (198 liters). In addition, coated sand column initially removed greater than 99.99% of rotavirus SA11 from seeded water (Table 7). RT-PCR analysis and cell culture infectivity assays of the column Inffluents and effluents indicated similar removal efficiencies.

The Removal of Microorganisms by Columns Containing 35 Mesh Sand

The removal of different enteric viruses and bacteriophages by columns (5cm x 25cm) containing 35 mesh sand coated according to method 2 are shown in Table 8. The coated sand columns removed 90-99% of the different bacteriophages that were added to the water. These columns reduced the number of the various challenge enteric viruses by roughly the same magnitude according to the cell culture methods. RT-PCR analysis of the column Inffluent and effluents indicated similar removal efficiency for polio virus 1 and rotavirus SA11 (Table 9). Additionally, These column removed over 99% of the seeded *Cryptosporadium parvum* oocysts, whereas untreated sand columns removed about 40% (Table 10). Similar reductions were obtained by performing PCR on the columns influents and effluents for Cryptosporadium parvum HSP 70 gene.

Integrated cell culture PCR (ICC/PCR) also indicated similar reductions. However these tests were able to analyze larger sample volumes and indicated higher concentration of oocysts in the columns inffluent and effluent. Rt-PCR of the *Cryptosporadium parvum* mRNA indicated similar reductions, but indicated lower concentrations of oocysts in the inffluent.

In separate experiments, when the column effluents were recirculated into the challenge tank, total removal of cell culture infectious units of polio, echo, and Coxsackie virus was obtained after 30 minutes of operation (Table 9). Viral nucleic acids were also undetectable. Integrated cell culture (IC) RT-PCR gave the same result as did the cell culture methods. However, IC/RT-PCR indicated the presence of enteroviruses in 36 hours, while the cell culture methods required approximately 48-96 hours to obtain a positive result.

Microorganism Removals by Swimming Pool Filters

At the beginning of the study, both one pass experiments and 1 hour water recirculation tests were performed (Table 11). After one pass through the filter, the average removal of all viruses by the filter containing untreated sand filter was just above 20%. In contrast, after one pass through the filter with modified sand, the average viral removal of all viruses was over 60%. *Cryptosporidium parvum* oocysts were reduced by 51% by filters containing coated sand. By allowing the water to circulate through the filter for 1 hour, the average removal of all viruses was over 99.5% by the filter containing coated sand. *Cryptosporadium* oocysts were reduced by an average of 95%. The filter

with untreated sand removed approximately 40% of the test viruses and 36% of the oocysts. When the filters were challenged with bacteria, an average removal of 78% was obtained with the filter containing modified sand. In comparison, the filter with the unmodified sand removed 40% of the bacteria. The percent removal of various microorganisms did not drop significantly over the length of the test period. Figure 9 shows the average viral removals at day 1, 29, 68, and 170. Each point represents an average reduction of values obtained for MS-2, PRD-1, ϕX 174, and Polio 1. At day 170 the filter containing modified sand reduced the number of bacteriophage by 2.4 $\log_{10}$ units as compared to 3.1 $\log_{10}$ unit reduction at the beginning of the test period. The microorganism removal by untreated filters was constantly under 0.2 $\log_{10}$ unit reduction.

Virus Removals by Tap Water Faucet Filters Containing Coated Solids

The presence of coated sand and diatomaceous earth in the tap water faucet filters greatly enhanced the viral removal efficiency of these filters (Figure 10). Initially the filters containing coated solids removed polio1 by >99%, MS-2 by 95%, ϕx174 by 95%, and PRD1 by >99%. At the end of the 28 day challenge period the efficiency dropped to 95%, 93%, 62%, and 92% respectively. The removal of these viruses was substantially lower by the filters containing uncoated sand or diatomaceous earth. Initially, the filters removed polio1 by 16%, MS-2 by 17%, ϕx174 by 11%, and PRD1 by 12% (data not shown). The viral removal at the end of the test period slightly rose to 28%, 21%, 19%, and 27%, respectively (data not shown). No significant differences were noticed in

the performance of the filters when they were kept at room temperature or at 4°C (data not shown).

Water Metal Analysis

All column, swimming pool filter, and faucet filter samples contained less than 0.01 ppm iron or aluminum. Even when water was recirculated through the filters numerous times, iron and aluminum were below the detectable levels of the instrument used.

Discussion

For the past ten years, our lab has been studying the modification of solids for the enhanced removal of microorganisms from water. One of the goals of these studies is to produce filters that can be used to remove microorganisms from water during treatment and therefore reduce the amount of disinfectant required to produce safe water. Another goal of these studies, is to produce point of use filters that can remove microorganisms in the event of a failure in the disinfection process. Metallic hydroxide floc's ability to adsorb microorganisms has been useful in treating and in recovering viruses from water. Aluminum and ferric hydroxides are frequently used in water treatment processes to reduce turbidity and remove microorganisms. They are also used on a smaller scale in microbiological laboratories for the concentration and detection of microorganisms (Bitton, 1994; Daniels, 1980; Farrah, 1978; Gerba, 1984; Goldstein, 1992). Farrah et al. (1988, 1991) previously demonstrated that

metallic hydroxides and oxides can be directly precipitated onto the surface of the solids by the process of in situ precipitation. I have previously shown that solids coated with metallic hydroxides adsorb viruses and bacteria efficiently from water (Lukasik et al., 1996; Lukasik, 1997). Other researchers have examined the use of these solids in water treatment processes (Mansoor Ahmed and Chaudhuri 1996). In this Chapter, I determined the effect of using the different developed coating methods on the removal efficiency and the durability of the modified solids. I also explored the use and determined the pathogen removal efficiency of coated sand or diatomaceous earth in columns, swimming pool filters, and facet filters in removing pathogenic microorganisms from water. Previously established methods and novel techniques were used to evaluate the removal efficiency of the coated solids. This was performed in order to determine the most sensitive and effective means for detecting the various pathogens from water samples for future studies.

The different tested coating methods gave relatively similar viral removal efficiencies. However, sand coated according to method 1 lost almost half of its original removal efficiency after it was subjected to rigorous shaking. The removal efficiency of sand coated according to the other methods did not fall that drastically. On the average, the performance of sand coated according to method 2,3, 4, and 5 dropped to about 75% of its value before attritioning. Even though, method 3 generates a slightly more efficient coating on the solids, it was not further used due to its lengthy coating process. Method 3 and 4 coated solids as efficiently as did method 2 and took only 30 minutes to perform. Where

as method 2 required 24-48 hours. However, for further studies method 2 was chosen since large quantities of solids can be conveniently coated by this method at the same time.

Large (5x25cm) columns containing 500ml of coated 35 mesh sand removed 90-99.5% of the different challenge viruses. The removal of Echo virus 3 and Rotavirus SA 11 was 90.6 and 96.5%, respectively. These viruses usually have the lowest removals in filtration experiments. Uncoated sand removed less than 25% at all times. The different methods that were employed for the detection of *Cryptosporadium parvum* oocysts indicated a removal greater than 99% when an inffluent containing 100-500 oocysts/ml was passed through the columns containing coated sand. The Immuno flourescent assay (IFA) method of oocyst determination was painstaking to perform but had the advantage of accommodating large sample volumes. PCR analysis of the column inffluent and effluent indicated substantially lower oocyst concentrations. This indicated that this method has a ten fold lower sensitivity than did the IFA method. Both the IFA method and the PCR method are unable to differentiate between the detection of viable or inactivated oocysts. To over come that problem, cell culture integrated PCR and Rt-PCR of the oocyst mRNA was used. These methods were more sensitive for the detection of oocysts than the direct PCR method since they indicated the presence of higher concentrations than did the PCR method. However, they indicated a lower concentration of oocysts in the samples assayed than did the IFA method (about half as many oocysts were detected). This is due to the fact that these methods detect viable oocysts

where as the IFA methods does not distinguish between live or dead oocysts. The Rt-PCR of the mRNA proved to be either, more selective for detecting live oocysts or less sensitive for detecting the oocysts than did the ICC-PCR.

Recirculating the pathogen seeded water numerous times (54 bed volumes) through the columns containing coated sand, resulted in a greater than a 99.999% reduction of the test viruses. Viral nucleic acids were undetectable in the column effluents when tested for by RT-PCR. IC/RT-PCR resulted in comparable results for viral removals as did the cell culture methods. However, the IC/RT-PCR gave results in 48 hours, whereas the cell culture methods required at least 96 hours to perform. On the other hand, uncoated sand columns barely removed about 82% after the passage of 324 bed volumes of water when assayed by cell culture. RT-PCR analysis of the uncoated sand column influents and effluents throughout the 2 hour test period indicated a reduction of about 50%. Whereas, IC/RT-PCR results indicated a 94% reduction in the numbers of viruses. These results indicate that the RT-PCR by it self does not provide an absolute alternative to the cell culture methods since the presence of an actual infectious virus is not certainly determined. This method does not differentiate between live or dead viruses. It may be also that naked viral RNA is being detected and amplified. Thus, explaining the persistence of a positive RT- PCR after three hours or recirculation. In addition, the sample volume assayed (ten micro liters) is so small that accurate determinations of the numbers of viruses are hard to perform. The combination of cell culture and RT-PCR gives the advantage of the detection of only viable viruses. This methods

also allows the examination of larger sample aliquots and provides results quicker than the conventional cell culture techniques.

The use of coated 100 mesh sand in comparable column resulted in far better removals. Removals greater than 99.99% were initially obtained. After the using the columns for 30 days polio removal was still higher than 99.9%. After 48 days of use the removal of polio 1 by coated sand columns was comparable to that of uncoated sand columns. The removal of MS-2 and SA-11 was over 99.99% initially and only dropped to about 99% at the end of the experiment. In general, the viral removal of the uncoated sand columns increased with time and water passage, whereas the efficiency of coated sands decreased. The most likely cause of this trend is the colonization and formation of biofilm on the surface of the sand. Examination the coated sand surface after prolonged exposure to tap water by a scanning electron microscope revealed numerous microorganisms that covered the surface of the coated sand (Figure 11). This colonization of the surface masks the sites that are available for viral adsorption. However, viral adsorption does occur to the surface of the new biofilm, hence the improved removal efficiency of the uncoated sand. By coating swimming pool filter sand with metallic hydroxides, two to three fold improvements in microbe removals were obtained. This improvement was consistent throughout the test period. Higher removals (99.9% for viruses and 95% for *Cryptosporadium*) were obtained when water containing the microorganisms was circulated through the filter numerous times. The percent removals did not vary when the experiments were performed at different times.

However, a slight drop in the percent removal was observed for the modified sand filter at the end of 170 day test period. The combined percent reduction for the viruses was still above 99.5%.

Faucet filters containing modified solids removed greater than 95% of the viruses added to tap water. At the end of the 28 day study these filters were still able to remove more than 85% of most of these viruses.

In conclusion, in all cases studied filters and columns containing particles of method 2 coated solids removed significantly more microorganisms than did filters and columns composed of uncoated particles. The incorporation of metal hydroxide coated solids greatly enhanced the performance of the filter systems tested. The use of filters with coated solids may not provide an absolute alternative to disinfection but may reduce the amount of disinfection required. They may also reduce disease transmission by providing an additional safety barrier. This could be especially important if there is an interruption or failure in the disinfection process. Based on this and previous studies, I believe that in situ precipitation of metallic hydroxides is a relatively easy and inexpensive method for modifying solids. The resulting coatings of metallic hydroxides are stable for long periods and are able to efficiently remove microbiological contaminants from water.

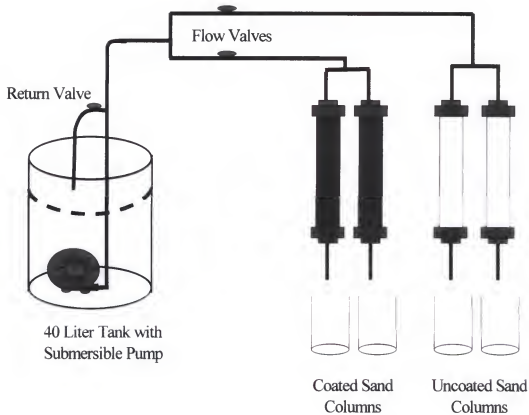

Figure 4. Schematic of the column filter set up used in viral removal experiments

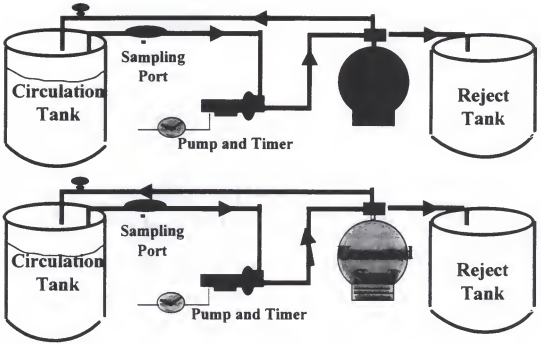

Figure 5. Schematic of the swimming pool filter set up described in the Materials and Methods section

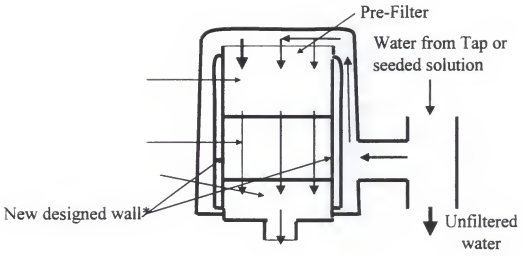

Figure 6. Schematic of the faucet filter that was used in viral removal experiments.

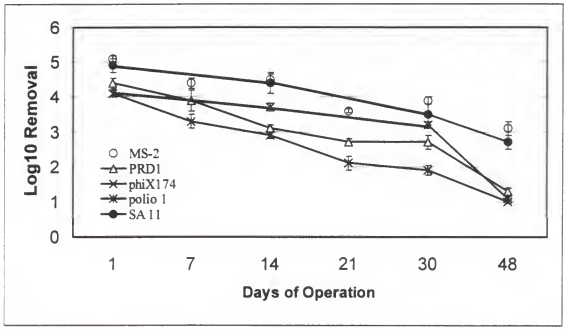

Figure 7. Removal of viruses by columns (35.5 X 5.0 cm) containing 1 kg of 100 mesh sand coated according to method 2.

Note: Four liters of water was passed through each of these columns daily. At the different days the indicated viruses were added to buffered pH 7 water to obtain an average concentration of 10^5 pfu/ml. The water was passed through the columns at 400 ml/min. The effluent was collected and the viral concentration was determined.

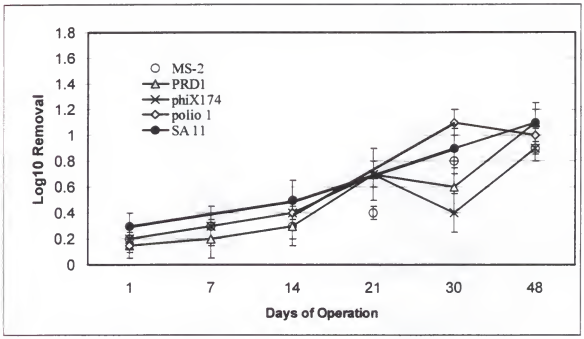

Fig 8. Removal of viruses by columns (35.5 X 5.0 cm) containing 1 kg of 100 mesh uncoated sand.

Note: Four liters of water was passed through each of these columns daily. At the different days the indicated viruses were added to buffered pH 7 water to obtain an average concentration of 10^5 pfu/ml. The water was passed through the columns at 400 ml/min. The effluent was collected and the viral concentration was determined.

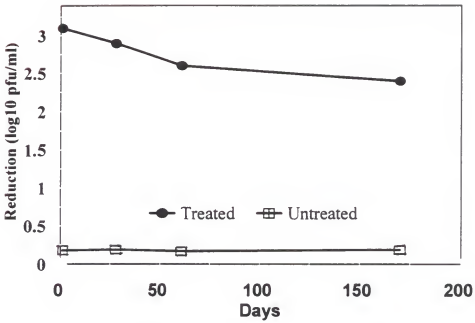

Figure 9. Removal of viruses from water by filters containing modified or unmodified sand (recirculation mode).[1]

[1] Each data point represents the average of viral removals. In all cases, triplicate samples were assayed and the differences between removal by filters containing unmodified sand and filters containing modified sand were statistically significant ($p < 0.05$). The error associated with every data point was less than 10%.

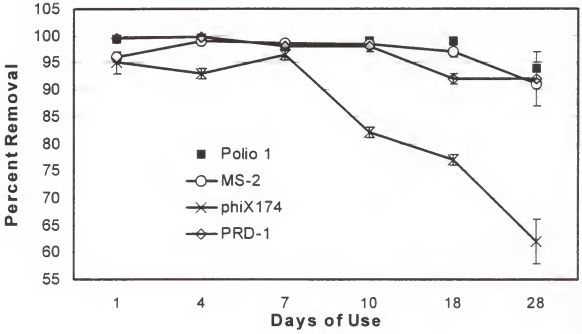

Figure 10. Removal of microorganisms by faucet filters containing coated solid

Note: Ten liters of water was passed through each of these filters daily. At the different days the indicated viruses were added to buffered pH 7 water to obtain an average concentration of 10^5 pfu/ml. The water was passed through the columns at 100 ml/min. The effluent was collected and the viral concentration was determined.

Figure 11. Scanning Electron Micrograph of the surface of coated sand from a column that had water passed through it for 30 days.

Table 5.Sequences of the Primers Used for the detection of Enteric Viruses and *Cryptosporidium parvum*

Enteric Virus	Primer Pair (5'-3')	Product Size
Polio 1	CGTTATCCGCTTATGTACTT CAGCAAACAGATAGATAATG	396
Pan Enterovirus	CCTCCGGCCCCTGAATG ACCGGATGGCCAATCCAA	197
Rotavirus		
Pair 1	GTCACATCATACAATTCTAATCTAAG TGTATGGTATTGAATATACCAC	1011
Pair 2	GTCACATCATACAATTCTAATCTAAG ACTGATCCTGTTGGCCAWCC	345
Cryptosporadium parvum	AAATGGTGAGCAATCCTCTG CTTGCTGCTCTTACCAGTAC	361

Table 6. Removal of viruses by 35 mesh sands coated according to different methods previously described before or after attritioning.

Challenge Virus	Log_{10} Removal of Seeded Viruses By									
	Sand Coated According to Method:					Attritioned Sand Coated According to Method:				
	1	2	3	4	5	1	2	3	4	5
MS-2	2.4	2.7	2.9	2.7	2.6	1.4	2.0	2.1	2.0	1.9
ϕX174	1.9	2.2	2.3	2.3	2.1	0.9	1.6	1.8	1.5	1.5
PRD1	2.3	2.5	2.6	2.3	2.4	1.2	1.7	1.9	1.8	1.7
polio 1	2.4	2.8	3.0	2.8	2.7	1.5	2.1	2.2	2.0	2.0

Note: Adsorption experiments were conducted in buffer at pH 7 using 40 g of 35 mesh sand coated sand as described in the text. The difference in viral adsorption between attritioned sand coated according to method 1 and the other methods were statistically different (probability >0.05)

Table 7. Comparison of RT-PCR and Cell culture for determining the removal of Rotavirus from water by columns containing Coated or uncoated 100 mesh sand.

Procedure	MPN (virus/ml) of:		
	Column Inffluent	Coated sand Effluent	Uncoated sand effluent
CPE on Cell Culture	4.6×10^5	4.6×10^1	4.1×10^5
Seminested RT-PCR	1.1×10^6	2.8×10^1	1.5×10^5

Note: Rotavirus SA11 was added to 20 l of dechlorinated buffered water. The water was passed through the columns at 400 ml/min and the effluent was collected. The most propable number of viruses in the inffluent and effluent was determined according to indicated methods and the corresponding percent removals were determined.

Table 8. Removal of viruses from water by columns containing coated (method 2) or uncoated 35 mesh sand.

	Percent Removal	
Challenge Microorganism	**Coated sand**	**Uncoated Sand**
MS-2	99.1 ± 0.1	13 ± 3
PRD-1	94.3 ± 0.8	11 ± 4
ϕX 174	91.5 ± 2	8 ± 3
polio 1	94.8 ± 3	11 ± 2
Echo 1	98.5 ±0.6	14 ± 4
Echo 3	90.6 ± 3	7 ± 2
Coxsackie B3	95.1 ± 2	9 ± 2
Coxsackie B5	97 ± 3	9 ± 3
rotavirus SA11[1]	96.5 ± 2	12 ± 2

[1] As determined by cytopathic effect observations (standard methods)
Note: Each column was 5x25cm and contained 500 ml of sand. Viruses were added to buffered pH 7 water to obtain an inffluent virus concentration of 10^5 pfu/ml. The water was passed through the columns and the effluents were collected and analyzed. The flow rate through the columns was approximately 900 ml/min.

Table 9. RT-PCR and IC/Rt-PCR of dilutions of water seeded with enteric viruses and recirculated through columns containing coated (method 2) or uncoated 35 mesh sand [1].

Sand	Min.	pfu/ml[1]	MPN as detemined by RT-PCR	MPN as determined by ICC/RT-PCR
Coated Sand	Initial	$5x10^5$	$5.0x10^5$	$1.0x10^6$
	0	$2.5x10^4$	$4.3x10^4$	$x10^5$
	30	0	<3.6	$3.6x10^{-1}$
	60	0	<3.6	$<3.6x10^{-1}$
	90	0	<3.6	$<3.6x10^{-1}$
	120	0	<3.6	$<3.6x10^{-1}$
	150	0	<3.6	$<3.6x10^{-1}$
	180	0	<3.6	$<3.6x10^{-1}$
Plain	Initial	$6x10^5$	$9.3x10^5$	$1.0x10^6$
	0	$4x10^5$	$2.8x10^5$	$2.4x10^5$
	30	$3x10^5$	$1.5x10^5$	$2.0x10^5$
	60	$1x10^5$	$4.3x10^5$	$9.4x10^4$
	90	$9x10^4$	$9.3x10^4$	$2.4x10^5$
	120	$9x10^4$	$4.3x10^5$	$7.5x10^4$
	150	$8x10^4$	$2.1x10^5$	$4.3x10^4$
	180	$7x10^4$	$9.3x10^4$	$4.3x10^4$

Note: Each column was 5x25cm and contained 500 ml of sand. Viruses were added to buffered pH 7 water to obtain an inffluent virus concentration of 10^5 pfu/ml. The water was circulated through the columns in a closed loop fashion and the effluents at each indicated time point were collected and analyzed. The flow rate through the columns was approximately 1 l/min. Dilutions were made and RT-PCR (or IC/RT-PCR) was performed.

[1] As determined by a plaque assay on RD cells

Table 10. The removal of *Cryptosporidium parvum* by columns (25x5 cm) containing 35 mesh coated sand.

Procedure	Oocysts/ml in Column Inffluent	Oocysts/ml in Column Effluent
IFA (counts)	150.3 ± 58	0.7 ± 1.2
PCR (MPN)	.7 ± 0.6	<0.25
ICC/PCR (MPN)	60.0 ± 16	<0.25
ICC/ RT-PCR (MPN)	23.0 ± 7	<0.25

Note: Each column was 5x25cm and contained 500 ml of sand. Buffered pH 7 water was seeded with *Cryptosporidium parvum* oocysts. The water was passed through the columns and the effluents were collected and analyzed by the different indicated detection procedures. The flow rate through the columns was approximately 1l/min. NP: not performed.

Table 11. Removal of microorganisms by Porpoise filters containing modified or unmodified sand. Both one pass experiments and water circulation through the filter experiments are shown.

Microorganism	Viral Removal by One Pass Filtration		Viral Removal by Water Circulation	
	Treated	Untreated	Treated	Untreated
SA 11	49%	23%	99.4%	38%
Echo 1	63%	25%	99.8%	35%
Polio 1	73%	27%	99.7%	39%
Coxsackie B5	79%	30%	99.9%	45%
MS-2	53%	17%	99.98%	34%
ϕX 174	69%	24%	99.9%	43%
PRD-1	45%	8%	99.8%	49%
E. coli	NP	NP	67%	35%
S. aureus	NP	NP	88%	44%

Note: Viruses were added to pH 8.3 water to obtain an inffluent virus concentration of 10^5 pfu/ml. The water was passed or circulated through the filters containing 0.05 m^3 coated or uncoated sand. Effluents were collected and analyzed. The flow rate through the filters was approximately 40 g/min. Data represent the average of triplicates. In all cases, the differences between removal by filters containing unmodified sand and filters containing modified sand were statistically significant ($p < 0.05$). NP: not performed. The error associated with the reported values was $< \sqrt{n}$ (n= the number of counts for each assay replicate)

CHAPTER 4
THE EFFECT OF SALTS ON THE REMOVAL OF VIRUSES FROM WATER BY COATED SOLIDS, UNCOATED SOLIDS, AND MICROPOROUS FILTERS.

The effect of salts on virus adsorption to microporous filters has been studied for several years (Gerba, 1984; Mix, 1974; Mix, 1978; Payment et al., 1976; Wallis et al., 1972) and has been discussed in many reviews. In these studies, researchers concluded that the addition of aluminum ions (or magnesium ions) enhanced viral adsorption to the filters, and from these results it was concluded that the presence of salts was necessary for optimum viral adsorption to filters (Wallis et al. 1979). However, we found two problems with the earlier studies:

First, relatively few viruses and few microporous filters were studied. Much of the information was on the adsorption of polio virus to nitrocellulose filters (Millipore). However, due to recent developments in viral filter development, new filters are currently being used that have substantially different properties from the ones previously studied. Therefore, it is necessary to evaluate the effect of salts on these filters.

Second, studies on the effect of aluminum salts on virus adsorption did not distinguish between direct and indirect effects of the salts that influenced

viral adsorption. The indirect effects include: (1) lowering of the pH by the addition of aluminum salts to purified water (Wallis et al., 1972); (2) the formation of virus adsorbing flocs that are subsequently physically trapped by the filters(Farrah et al., 1978); (3) and the reaction between aluminum ions and the humic materials that interfere with virus adsorption (Farrah et al., 1976; Sobsey and Glass, 1980).

In order to achieve a better understanding of the forces involved in viral adsorption to solids, I investigated the adsorption of four viruses to four commercially available filters with different compositions and different physical characteristics. We also evaluated the effect of salt solutions on the adsorption to iron and aluminum hydroxide coated diatomaceous earth. Mono and multivalent salts were used at different pH values and compounds that have been shown to disrupt hydrophobic interactions were also used. In addition, the effect of different concentrations of aluminum or magnesium on the pH, and respectively viral adsorption, was studied.

The previously described direct and indirect effects were minimized through the control of flocculation and pH by filtering and buffering. All virus stocks and salt solutions were pre-filtered through 0.2 µm porosity filters prior to each experiment to reduce the effects of aggregation and flocculation. The pH was monitored and controlled and only purified water was used.

Materials and Methods

Viruses

The following phages and their hosts were used: MS2, *Escherichia coli* C-3000 (ATCC 15597); ϕX-174 and *E. coli* (ATCC 13607); and PRD-1, *Salmonella typhimurium* (ATCC 19585). Phages were assayed as plaque-forming units (PFU) using their respective hosts and a soft-agar overlay (Snustad and Dean 1971). Polio virus Lsc 1 was grown on and assayed as PFU on BGM cells using an agar overlay method (Smith and Gerba 1982). Before there use, all viral stocks were prefiltered through a 0.2-µm porosity filter that had been prerinsed with 10 ml of deionized water (Millipore GS membrane, Millipore Corp., Bedford. MA).

Microporous Filters

The following microporous filters were used: Millipore HA (nitrocellulose, Millipore Corp., Bedford, MA); Filterite 0.22-µm (bonded fiberglass, Filterite Corp., Timonium Md.); Whatman # 5 (cellulose, Fischer Scientific, Pittsburgh, PA); and 1MDS (charge-modified fibers, AMF Cuno, Meridan CT). The filters were cut into 25 m.m. circles and placed into a 25 m.m. stainless steel filter holder (Fischer Scientific, Pittsburgh, PA)

Chemical Solutions

Solutions of sodium chloride, magnesium chloride, aluminum chloride, urea, and Tween 80 were made to the indicated concentrations in a buffer solution (0.02 M immidazole + 0.02 M glycine, unless otherwise indicated). The solutions were adjusted to the required pH by the addition of 1 M NaOH or 1 M HCl. All solutions were prefiltered through Millipore 0.2-µm GS (Millipore Corp., Bedford, MA) filters that had been pre-rinsed with deionized water.

Aluminum Titration Experiments

For aluminum titration experiments, aliquots of a 1.00 M aluminum chloride solution were added to a 0.002 M glycine and 0.002 M immidazole solution to obtain the desired concentrations. The solutions were prefiltered through a Millipore 0.2-µm GS filter and the pH of the solutions were measured. In addition, solutions of the above buffer were made up and adjusted with HCl to a pH that matched the pH of the aluminum solutions. The pH of these solutions were measured at the beginning and end of all experiments.

Experimental Procedure

Half a gram of coated (method 2 described in chapter 2 and 3) or uncoated diatomaceous earth was packed into filter holders containing a cellulose filter support as shown in figure 12. Microporous filters were cut into 25mm circles and placed into stainless steel filter holders. One layer of Millipore HA, two layers of Filterite, two layers of Whatman 5, or three layers of 1MDS

were placed in each holder for use in the experiment. Before performing the experiment, each filter was first rinsed by passing sixty milliliters of deionized water. Viruses were added to solutions to produce an initial titer of approximately 10^5 PFU/ml. Viruses in the solutions were assayed following dilution in 1% Tryptic Soy Broth (Difco) for phages or MEM with 2% FCS for polio virus. Dilution was sufficient to raise the pH of samples to approximately 7. To prevent flocculation when conducting experiment with aluminum chloride, an initial 1/10 dilution into deionized water was made first, then a second dilution into the dilution media described above was made. Twenty-five ml of the salt solution containing the viruses were passed through each of the filters by a mechanical syringe infusion pump (Harvard Apparatus Co. Millis, MA) at a flow rate of 1.5 ml/sec. The filter's effluent was assayed for viruses and percent removals were determined . A portion of each sample that had not been passed through the filters was assayed at the beginning and at the end of the experiment to determine if the solutions inactivated the viruses. The average of these numbers was then used to determine the percent removal by the filters. All filter experiments were performed at least twice and in triplicates each time. Samples from each experiment were plated in triplicates. The statistical analysis of the obtained data (Standard deviations, slopes, correlation and general t test probabilities) were determined using PSI-Plot software (Poly Software International, Salt Lake City, Utah).

Results

Effect of Salts on the Adsorption of Viruses to Microporous Filters

The effect of salt solutions at pH 7 on the adsorption of the viruses studied to the filter is dependant on the filter type and the ionic species (Table 12). In general, the addition of salts increased the adsorption of viruses to Millipore filters and interfered with the adsorption to 1 MDS filters. They did not affect the viral adsorption to Whatman filters, but did slightly increase the adsorption to Filterite (especially polio 1).

At pH 3.5, none of the salts affected viral adsorption to Millipore or Whatman filters (Table 13). More than 90% of the viruses tested absorbed to Millipore filters and less than 10% adsorbed to Whatman filters under all conditions tested. However, the salts interfered with viral adsorption to 1MDS and Filterite filters. The degree of the interference depended on the virus and the salt used. Aluminum chloride interfered with adsorption of viruses to 1MDS filters more than did magnesium chloride or sodium chloride. Only aluminum chloride interfered significantly with virus adsorption to Filterite filters.

The differential effect of salts on virus adsorption filters is shown in Figures 13, 14 and 15. At pH 7, increasing the concentration of magnesium chloride increased polio virus 1 adsorption to Millipore HA filters but decreased its adsorption to 1MDS filters (Figure 13). At pH 3.5, increasing the concentration of magnesium chloride (Figure 14) or aluminum chloride (Figure

15) did not affect adsorption of polio virus 1 to Millipore HA filters, but decreased its adsorption to 1MDS filters.

At pH 7 and in the absence of salt ions, little adsorption of polio virus 1 to Millipore HA filters was observed. As the concentration of aluminum chloride was increased, the adsorption of polio virus was also increased (Figure 16). The pH of the solutions also decreased as aluminum chloride was added. When the pH of the solutions were decreased by the addition of HCl, a similar trend in virus adsorption was observed. The decrease in the pH of the solution caused by the addition of aluminum chloride may explain the previously reported high viral adsorption associated with the addition of aluminum chloride to unbuffered water.

The addition of urea in the absence of salt ions did not have a significant effect on viral adsorption to the filters [except for ϕX-174 where 4 M urea decreased the adsorption to the filters (Table 14)]. However, viral adsorption was reduced in the presence of both 0.1 M salt and 4 M urea at pH 7 or 3.5 (Table 15) The same effect was achieved in the presence of 0.1 M salt and 0.1 % Tween 80.

The effect of prefiltering the salt solutions before adding the viruses is shown in Table 16. Basically, if a 0.0001 M aluminum chloride solution was made at pH 7 and viruses were added immediately to that solution and were passed through a Millipore HA filter, then good viral removals were obtained. If that aluminum solution was passed through a 0.2 μm GS filter first, then the

viruses were added to that solution and it was passed through the Millipore HA filter, then no significant viral adsorption was observed.

Effect of Salts on Viral Adsorption to Coated and Uncoated D.E.

As seen in Table 17, at pH 7, little viral adsorption occurred to uncoated diatomaceous earth (D.E.). At that same pH, significant (>99%) adsorption occurred to coated sand. The addition of magnesium chloride (final concentration 0.1 M) to the filter influents promoted the adsorption of polio1 to uncoated diatomaceous earth at pH 7. It did not promote the adsorption of the other viruses to the diatomaceous earth. However, the addition of urea (final concentration 4M) to the inffluent containing the magnesium chloride eliminated the adsorption of polio 1. The presence of urea by itself has no effect on viral adsorption. The presence of magnesium chloride interfered with the adsorption of phiX174 to the coated diatomaceous earth. It did not drastically affect the adsorption of the other viruses. At pH 3.5 viruses adsorbed fairly well to the coated or uncoated diatomaceous earth in the presence of buffer alone. The addition of aluminum chloride reduced the adsorption of polio 1 and phiX174 to uncoated diatomaceous earth. The addition of both magnesium chloride and urea (or Tween 80) interfered with the adsorption of all the tested viruses. Similar results are obtained when coated diatomaceous earth was used.

Discussion

Effect of Salts on the Adsorption of Viruses to Microporous Filters

Based on early studies on the influence of salts on virus adsorption, it was suggested that salts promoted virus adsorption to microporous filters by promoting electrostatic interactions between the virus and the filter. The possible mechanisms that were suggested to explain these observations were salt bridging or charge neutralization of the filter. It was also suggested that the ability of the salts to promote virus adsorption was related to the valence of the cation. Trivalent cations (Al^{+++}) were better than divalent cations (Mg^{++}), which were better than monovalent cations (Na^{+}) at promoting virus adsorption when used at the same concentration. It was also suggested that solutions of metal chelators, such as EDTA or citrate ion, would elute viruses adsorbed to microporous filters. It was thought that the chelators would bind metallic ions that were involved in salt-bridging or charge neutralization. However, experiments have shown that EDTA and citrate can promote virus adsorption to hydrophobic microporous filters and interfere with adsorption to electropositive solids (Bitton, 1980; Gerba, 1984; Mix, 1974; Mix, 1987; Wallis et al., 1979).

In examining the early studies on the effects of salts on virus adsorption, we found three main problems. These are: 1. The role of salts on promoting hydrophobic interactions was not always considered; 2. Indirect effects of adding salts on the pH of solutions and the flocculation of these salts was not

always determined; 3. Few viruses and few filter types were studied. I would like to discuss these problems in relation to the current presented work.

(1) Hydrophobic interactions

The earlier studies on virus adsorption to microporous filters led to the suggestion that metal chelators would interfere with virus adsorption promoted by salts (Mix, 1974; Wallis et al., 1972). Later studies showed that chelators such as the citrate ion actually promoted, rather than interfered with, virus adsorption to microporous filters (Farrah, 1982; Farrah et al., 1981). These and other studies led to the demonstration that increased virus adsorption to certain microporous filters in the presence of cations and anions was influenced by hydrophobic interactions. Therefore, the adsorption of MS2 and polio 1 in the presence of salts to filters was best explained by the presence of hydrophobic interactions between the filter and the virus (Sheilds and Farrah, 1983). The role of added magnesium chloride on virus adsorption was then to strengthen hydrophobic rather than electrostatic interactions. In this study, urea and/or a neutral detergent (Tween 80) had little effect on virus adsorption. These compounds did not interfere or promote the adsorption of viruses to the filters tested at pH 7.0 or 3.5. In contrast, they greatly reduced virus adsorption when salts were present. Therefore the previous argument stands corrected, the salts were likely promoting hydrophobic interactions and interfering with electrostatic interactions between the viruses and the filters.

2. Indirect effects

The addition of salts to solutions can change the pH of a solution, cause floc formation, or interact with the organic material present in the solution. This is especially true for aluminum salts. Therefore, determining the direct role of aluminum ions on virus adsorption becomes difficult. As shown in figure 15, the result of increasing aluminum chloride concentration on virus adsorption reported by Wallis et al. (1972) can be explained by the effect of aluminum chloride on the pH of the solution. Lowering the pH of a solution, by adding either aluminum chloride or an acid, gave similar results on virus adsorption. It was not possible to reproduce the exact same effects of different concentrations of aluminum chloride on virus adsorption reported by Wallis et al. (1979), since these authors did not report the pH of the solutions used. However the similarities are substantial, and the trend is identical.

The addition of aluminum chloride (or magnesium chloride) to tap water or other solutions can produce flocs (Farrah et al., 1978). Removal of these flocs from water may also remove viruses and can be used to concentrate viruses from tap water. Previously, authors (Farrah et al., 1978; Wallis et al., 1972) have reported on the removal of viruses in tap water following addition of aluminum chloride. This is more than likely to be the result of the entrapment of flocs during filtration. As seen in Table 16, good viral removal is obtained if the pH 7 aluminum chloride solution (0.01 M) containing viruses is passed through a Millipore filter. That removal is lost when the solution is prefiltered and viruses are added, and then passed through the filter. Another indirect effect is the

forming of complexes with humic materials in water by the added cations(Farrah et al, 1976; Sobsey and Glass, 1984). This may explain the requirement for the addition of aluminum chloride to water in virus concentration procedures (Berg et al., 1984). It may also explain why higher concentrations of aluminum chloride are required for recovering viruses from organic-rich estuarine waters (Payment et al., 1976) than from the cleaner tap water.

3. Viruses and filters studied

In much of the earlier work, adsorption of polio virus and MS2 to Millipore HA filters was studied. These studies led to the conclusion that salts promote virus adsorption to microporous filters. In this study, we used three filters that are relatively electronegative at pH values near neutrality (Millipore, Filterite, and Whatman) and one filter that is more electropositive under the same conditions.

As shown in this study, salts can promote virus adsorption, interfere with virus adsorption, or have little effect on virus adsorption. We believe that the theories on the effects of salts on virus adsorption should account for these multiple effects.

From the observed results we conclude that the presence of salt cations promotes hydrophobic interactions between the virus and the filter or substrate. These salt cations also interfere with the electrostatic interactions that might occur between the virus and the filter. Electrostatic interaction cause viral adsorption when an electropositive filter surface is present and viruses are above their isoelectric points. They may also cause adsorption when an electronegative filter surface is present and viruses are below their isoelectric

point. It is our conclusion also that urea and Tween 80 can be used to disrupt hydrophobic interactions. The presence of both Urea (or Tween 80) and multivalent salt is likely to overcome most of the forces responsible for viral adsorption to the microporous filter surfaces.

Effect of Salt Solutions on Viral Adsorption to Coated or Uncoated D.E.

The mechanisms involving the adsorption of viruses to the modified diatomaceous earth appear to be predominantly electrostatic in nature. Multivalent salts were capable in disrupting some of the viral adsorption to coated solids. However, 4 M urea or neutral detergents (Tween 80) had no effect on viral adsorption to coated solids by their self.

The adsorption to untreated diatomaceous earth is slightly more complicated. The addition of salts increased the adsorption of polio 1 (and slightly MS2) to untreated diatomaceous earth at pH 7. This observation is best explained by the fact that the presence of salts strengthens the hydrophobic interactions to the diatomaceous earth. At pH 3.5, all the viruses are below their isoelectric point and therefore exhibit a net positive charge on their surface. This positive charge results in the adsorption of the viruses to the negatively charged diatomaceous earth. The addition of salt disrupts some of these interactions however the hydrophobic interactions still exist. The addition of urea or Tween 80 disrupts the hydrophobic interactions. Therefore in the presence of both urea and a multivalent salt viral adsorption can be minimized. These observation imply that, at pH 7 polio 1 virus has more hydrophobic interactions with the

surface of the solids than the other viruses. Accordingly, in the presence of a multivalent salt it adsorbs to the slightly hydrophobic surface of the diatomaceous earth. The bacteriophage phiX174 displays the opposite trends. From the results it seems that phiX174 adsorbed to solids via predominantly electrostatic interactions. In the presence of salts, these interactions are broken and the virus no longer adsorbs to the surface. In conclusion, polio 1 exhibits strong hydrophobic interactions, phiX174 exhibits strong electrostatic interactions, PRD1 exhibits week hydrophobic interactions, and MS2 seems to exhibit equally very strong electrostatic and hydrophobic interactions.

Table 12. Influence of salts on virus adsorption to microporous filters at pH 7.

Filter	Virus	Percent Virus Adsorption in Presence of:		
		Buffer	0.1 M NaCl	0.1 M $MgCl_2$
Millipore HA	MS-2	8	18	99
	PRD-1	6	8	59
	ϕX-174	4	40	57
	Polio 1	3	98	99
	Mean	**5**	**41**	**79**
Filterite	MS-2	5	21	23
	PRD-1	11	9	10
	ϕX-174	3	3	5
	Polio 1	10	9	99
	Mean	**7**	**11**	**34**
Whatman	MS-2	18	12	19
	PRD-1	7	3	6
	ϕX-174	5	9	38
	Polio 1	8	6	5
	Mean	**10**	**7.5**	**17**
1 MDS	MS-2	96	10	7
	PRD-1	97	13	13
	ϕX-174	29	12	18
	Polio 1	79	7	9
	Mean	**75**	**11**	**12**

Note: Viruses were added to 50 ml of buffer (0.02 glycine and 0.02 immidazole) alone or buffer with the indicated salt and then passed through 25 mm filter holders containing the indicated filters. The virus concentrations in the inffluent and effluent samples were determined and the corresponding percent adsorptions were calculated. The error associated with the reported values was $< \sqrt{n}$ (n= the number of counts for each assay replicate)

Table 13. Influence of salts on virus adsorption to microporous filters at pH 3.5.

Filter	Virus	Percent virus adsorption in Presence of:			
		Buffer	0.1 M NaCl	0.1 M $MgCl_2$	0.1 M $AlCl_3$
Millipore HA	MS-2	>99	>99	>99	>99
	ϕX-174	98	>99	>99	>99
	Polio 1	>99	>99	>99	>99
	Mean	**>99**	**>99**	**>99**	**>99**
Filterite	MS-2	>99	98	>99	96
	ϕX-174	95	97	98	23
	Polio 1	99	>99	97	10
	Mean	**98**	**98**	**98**	**43**
Whatman	MS-2	3	4	3	4
	ϕX-174	6	2	2	4
	Polio 1	9	7	5	7
	Mean	**6**	**4**	**3**	**5**
1 MDS	MS-2	95	60	44	18
	ϕX-174	85	75	73	15
	Polio 1	99	95	93	10
	Mean	**93**	**76**	**70**	**14**

Note: Viruses were added to 50 ml of buffer (0.02 glycine and 0.02 immidazole) alone or buffer with the indicated salt and passed through 25 mm filter holders containing the indicated filters. The virus concentrations in the inffluent and effluent samples were determined and the corresponding percent adsorptions were calculated. The error associated with the reported values was $< \sqrt{n}$ (n= the number of counts for each assay replicate)

Table 14. Influence of 0.1 M $AlCl_3$ or 0.1 M $MgCl_3$ and 4 M urea on virus adsorption to microporous filters at pH 7 and 3.5.

Filter	Virus	Percent Adsorption pH 7.0		Percent Adsorption pH 3.5		
		4M Urea	4M Urea+ 0.1M $MgCl_2$	4M Urea	4M Urea+ 0.1M $MgCl_2$	4M Urea+ 0.1M $AlCl_3$
Millipore HA	MS-2	6	1	98	15	13
	PRD-1	3	3	-	-	-
	ϕX-174	4	2	1	9	7
	Polio 1	7	2	>99	12	15
Filterite	MS-2	8	3	96	14	11
	PRD-1	6	2	-	-	-
	ϕX-174	3	1	1	7	8
	Polio 1	11	10	99	13	15
Whatman	MS-2	4	5	9	11	7
	PRD-1	4	4	10	9	5
	ϕX-174	5	7	4	3	6
	Polio 1	4	7	7	8	4
1 MDS	MS-2	96	6	95	14	8
	PRD-1	90	11	-	-	-
	ϕX-174	21	10	69	42	30
	Polio 1	68	8	99	9	10

Note: Viruses in 50 ml of buffer (0.02 glycine and 0.02 immidazole) alone or buffer with the indicated salt and/or urea were passed through 25 mm filter holders containing the indicated filters. The virus concentrations in the inffluent and effluent samples were determined and the corresponding percent adsorptions were calculated. The error associated with the reported values was < $\sqrt{n}$ (n= the number of counts for each assay replicate)

Table 15. Effect of salts and 4 M urea or 0.1% Tween 80 on viral adsorption at pH 3.5

Filter	Virus	Buffer	Percent Viral Adsorption in Presence of:			
			4M Urea + 0.1M MgCl$_2$	0.1% Tween 80+0.1M MgCl$_2$	4M Urea+ 0.1M AlCl$_3$	0.1% Tween80 +0.1M AlCl$_3$
Millipore HA pH 3.5	MS-2	>99	15	18	13	9
	PRD-1	-	-	-	-	-
	ϕX-174	98	9	10	7	5
	Polio 1	>99	12	19	15	17
1 MDS pH 3.5	MS-2	95	14	6	8	7
	PRD-1	-	-	-	-	-
	ϕX-174	85	52	80	30	41
	Polio 1	99	9	13	10	9
Filterite pH 3.5	MS-2	95	14	16	11	11
	PRD-1	-	-	-	-	-
	ϕX-174	95	7	5	8	5
	Polio 1	>99	13	11	15	14

Note: Viruses were added to 50 ml of buffer (0.02 glycine and 0.02 immidazole) alone or buffer with the indicated salt and Tween 80 or Urea. The solutions were then passed through 25 mm filter holders containing the indicated filters. The virus concentrations in the inffluent and effluent samples were determined and the corresponding percent adsorptions were calculated. The error associated with the reported values was < $\sqrt{n}$ (n= the number of counts for each assay replicate)

Table 16. The effect of prefiltering the aluminum solutions used in viral adsorption experiments to Millipore HA filters at pH 7.

Virus	Percent Viral Adsorption	
	Not Prefiltered	Prefiltered
MS-2	>99	13
PRD-1	98	8
ϕX-174	95	14
Polio 1	>99	16

Note: Two hundred milliliters of water was adjusted to 0.01 M aluminum chloride. Half of the solution was then prefiltered through 0.45 µm filters. Viruses were added to both solutions. The solutions were then passed through 25 mm filter holders containing Millipore HA filters. The virus concentrations in the inffluent and effluent samples were determined and the corresponding percent adsorptions were calculated. The error associated with the reported values was $< \sqrt{n}$ (n= the number of counts for each assay replicate)

Table 15. The influence of salts and urea on the adsorption of viruses to coated and uncoated diatomaceous earth at pH 7 and 3.5.

		Percent Viral Adsorption		
	Virus	Buffer	Buffer + 0.1M aluminum or magnesium chloride[1]	Buffer + 0.1M aluminum or Magnesium chloride + 4M urea [1]
Uncoated DE pH7	MS-2	8	23	5
	PRD1	10	8	8
	ϕx174	27	4	5
	Polio 1	15	>99	9
Coated DE pH7	MS-2	>99	>99	99
	PRD1	>99	>99	97
	ϕx174	>99	49	50
	Polio 1	>99	98	95
Uncoated DE pH 3.5	MS-2	>99	98	38
	PRD1	-	-	-
	ϕx174	>99	30	26
	Polio 1	>99	89	39
Coated DE pH 3.5	MS-2	>99	96	56
	PRD1	-	-	-
	ϕx174	95	10	10
	Polio 1	89	40	22

[1] Buffer in the presence of 4 M urea alone had no significant effect on viral adsorption as compared to the effect of buffer by itself. The error associated with the reported values was < $\sqrt{n}$ (n= the number of counts for each assay replicate)

Note: Viruses were added to 50 ml of buffer (0.02 glycine and 0.02 immidazole) alone or buffer with the indicated salt. The solutions were then passed through 25 mm filter holders containing 0.5 g coated or uncoated diatomaceous earth. Virus concentrations in the inffluent and effluent samples were determined and the corresponding percent adsorptions were calculated.

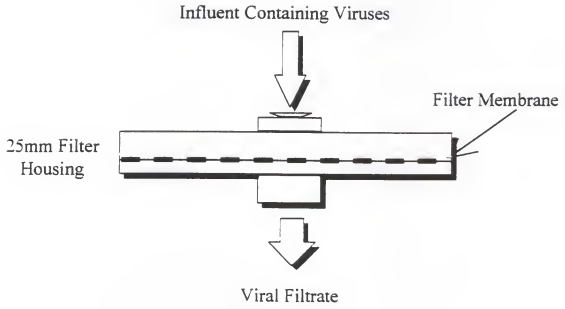

Figure 12. A schematic of the setup used to contain the diatomaceous earth for the adsorption interference experiments.

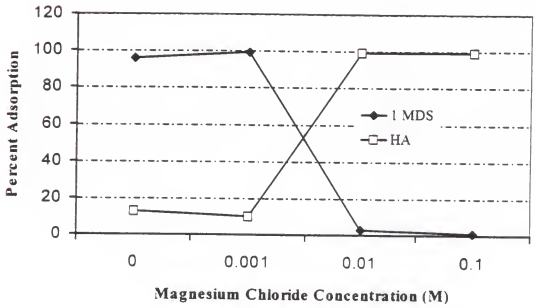

Figure 13. The effect of magnesium chloride concentration at pH 7 on the removal of polio 1 virus by 1 MDS and Millipore HA filters.

Note: Viruses added to 50 ml of buffer (0.02 glycine and 0.02 immidazole) adjusted to the indicated magnesium chloride concentrations were passed through 25 mm filter holders containing the indicated filters. The virus concentrations in the inffluent and effluent samples were determined and the corresponding percent adsorptions were calculated.

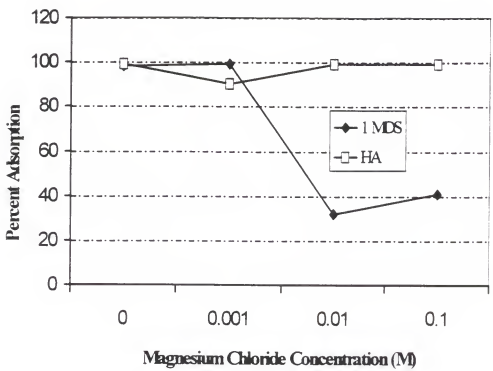

Figure 14. The effect of magnesium chloride concentration at pH 3.5 on the removal of polio 1 virus by 1 MDS and Millipore HA filters.

Note: Viruses added to 50 ml of buffer (0.02 glycine and 0.02 immidazole) adjusted to the indicated magnesium chloride concentrations were passed through the 25 mm filter holders containing the indicated filters. The virus concentrations in the inffluent and effluent samples were determined and the corresponding percent adsorptions were calculated.

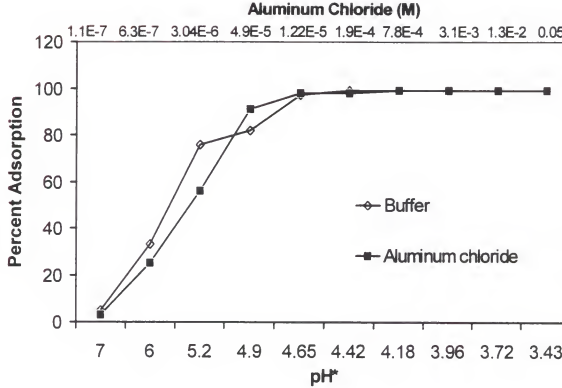

Figure 15. The effect of pH and aluminum chloride concentration on the adsorption of polio 1 to HA nitrocellulose filters.

Note: Aluminum chloride was added to purified water and the pH was recorded. HCl was added to purified water to produce the same pH value obtained fro the aluminum chloride addition. Polio virus was added to the solutions which were passed through 25 mm filter holder containing Millipore HA filters. The virus concentrations in the inffluent and effluent samples were determined and the corresponding percent adsorptions were calculated.

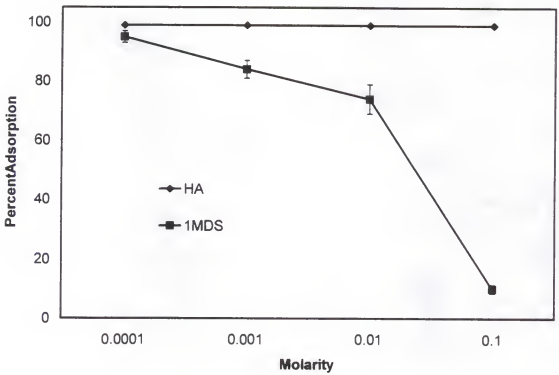

Figure 16. The effect of Aluminum chloride concentration on the adsorption of polio 1 to HA nitrocellulose filters at pH 7 and 3.5.

Note: Viruses added to 50 ml of buffer (0.02 glycine and 0.02 immidazole) adjusted to the indicated aluminum chloride concentrations were passed through the 25 mm filter holders containing the indicated filters. The virus concentrations in the inffluent and effluent samples were determined and the corresponding percent adsorptions were calculated.

CHAPTER 5
REMOVAL OF CHEMICAL CONTAMINANTS FROM AQUEOUS SOLUTIONS

The presence of many metals (especially heavy metals) in environmental waters can have detrimental effects on the living species in that environment. In addition, the presence of excessive concentrations of phosphates and organics in these waters also has deleterious effects on its quality. The growing industrial and agricultural activities of the modern world have led to the increased occurrence of environmental chemical contamination. This produces a potential health risk even when these chemicals are discharged in small concentrations. Metals are known to persist for long periods in the environment and accumulate in the food chain. Their presence in drinking water causes a variety of serious health problems that may lead to death in cases of extreme exposure (Volesky, 1990). An estimated 10% of the water borne disease outbreaks are attributed to chemical contamination. In addition, various heavy metals have been implicated as major contaminants in many superfund sites. Due to these concerns, strict standards have been set for the maximum allowed contaminant levels in discharged waters. Much attention has focused on the removal of these metals from water. Many processes have been suggested or developed to overcome that problem. These include: membrane filtration, biological filtration, chemical precipitation, electrode deposition, and ion exchange. Each of these processes

has its merits and limitations in application. Of the most popular water treatment processes, biological water treatment has been relied on for removing various chemical contaminants. However, this process results in a contaminated waste product that is relatively expensive to dispose of and metal binding also appears to be reversible (Zhang et al., 1998). Coagulated ferric and aluminum salts have been shown to remove many metallic impurities from water. However, constraints in the flocculation, sedimentation, filtration, and disposal processes have rendered chemical coagulation inefficient in removing low concentrations of heavy metals from large water volumes. Coating filter media (sand) with these precipitated salts might overcome some of these constraints. By doing so, this will provide the convenience of a flow through system that removes chemical contaminants from water. Previous researchers have studied the removal of metals by Fe(III) doped alginate gels (Min and Hering, 1998). This process is time consuming and not practical for treating large volumes of water. Sand coated with ferric oxides have been used to remove/recover trace heavy metals from water (Edward and Benjamin, 1989; Bailey et al., 1992; Stahl and James, 1991; Lo et al., 1994; Lai et al., 1994) The sand preparation and treatment process however required lengthy periods of time and the coating was not uniform throughout the sand surface. Kuan et al. (1998) studied the removal of selenite and selenate by aluminum oxide coated sand. The coating process was lengthy, but reasonable removals were obtained.

Previously, we have reported the use of ferric and aluminum hydroxide or oxide coated sand for the removal microorganisms from water (Farrah et al. 1991; Lukasik et al. 1996, Lukasik et al., 1998). From our results, better removals were obtained using precipitates of a combination of ferric and aluminum hydroxides. Viral removals greater than 99.9% were achieved. In this study, I determine the removal of metals from aqueous systems by columns containing sand coated with ferric and aluminum hydroxides. I also examine the composition of the sand after the initial challenge and after a 10 day period and evaluate the performance of the columns throughout a 43 day test period. In addition to the removal of metals, I also examine the removal phosphates and proteins from water by these columns.

Materials and methods

Modification of the Sand

The source of sand was " All Purpose Sand " from Pebble Junction, Division of Delaware Valley Landscape Stone, Inc., Sanford, Florida. Sand was sieved to 35 mesh before use. The sand was soaked in 0.25 M ferric chloride and 0.5 M aluminum chloride solution that was 50% ethanol. A sufficient amount of solution was used to completely cover the sand. After 10 minutes, the solution was poured off and the sand was allowed to dry at 85 °C with constant agitation. Next, the hot dry sand was gradually added to 2 volumes of 3 M ammonium hydroxide that was pre-warmed to 60 °C. The

ammonium hydroxide was poured off, the sand was dried again at 85°C. The dried sand was rinsed and stored at room temperature until used.

Column Adsorption Experiments

Coated or uncoated sand was packed into poly carbonate columns that were 25 cm tall and had an interior diameter of 5 cm. Five hundred milliliters (730 g) of sand was placed in each column. 80 mesh plastic screens were placed in the bottom of each column. Solutions were passed downward through the columns by a Little Giant pond pump (Little Giant, Okla City, OK) that was placed in a 40 liter tub. The flow rate was approximately 900 ml/min. Two columns contained coated sand and two columns contained uncoated sand. Eight liters of deionized water was initially passed through the columns to rinse the loose debris out of the columns. A schematic of the experimental set up is shown in Figure 4.

Synthetic Simulated Sewage Solutions

Synthetic simulated sewage solutions were prepared as previously described (Liu, 1997). Briefly, the substrates in Table 16 were added to deionized tap water in the given concentrations. A 10x stock solution of all the salts were prepared and used to constitute the working solutions. The stock solutions were kept frozen. Solutions of glucose and peptone were made at the time of use. Simulated sewage solutions containing these metals were used to challenge the columns in order to simulate the conditions encountered in waste water treatment processes.

Chemical Solutions

Stock solution of the following chemicals were made: cadmium chloride, cesium chloride, cobalt nitrate, cupric nitrate, chromium chloride, barium chloride, mercuric chloride, magnesium chloride, manganese chloride, lead nitrate, strontium chloride, silver nitrate, and zinc chloride. All the chemicals used were obtained from ACROS (ACROS Chemicals, Pitsburgh, PA). Aliquots of the above solutions were added to 20 liters of buffered tap water (adjusted to 0.002 M glycine and 0.002 M immidazole) or to synthetic sewage solution to achieve the desired concentration. The final concentration of these metals varied from 1-20 ppm.

Column Adsorption Experiments

Twenty five liters of tap water was passed through the columns containing coated or uncoated sand before each experiment. Aliquots of stock metal solutions were added to 20 liters of tap water or simulated sewage solutions. The solutions were passed through the column at a flow rate of 900ml/ min. and a superficial velocity of 0.82 cm/sec. Different metals were added either individually or in combination to the water or simulated sewage and were passed through the columns. Metal concentrations in the column influents and effluents were determined and percent removals for each column were calculated.

Batch Adsorption Experiments

Five grams of coated or uncoated sand was placed in a conical centrifuge tube. Twenty milliliters of water or simulated sewage containing one of the challenging chemical species was added. The tubes were agitated for 8 hrs. at room temperature by a slow rocking motion. The metal content of the solutions was measured before and after adsorption.

Complex Organic Matter and Dissolved Organic Carbon (DOC) Removals

Ten liters of water was obtained from Lake Alice (Gainesville FL) and prefiltered through a 0.45μm filter. The water was placed in the tub that was connected to the water pump and was passed through columns (25x5cm) containing 35 mesh coated or uncoated sand. Samples were taken out of the column inffluents and effluents. The DOC in these samples was measured by a TOC analyzer. Complex organic matter analysis was accomplished by measuring the adsorbance using a UV wavelength of 254 which corresponds to the peak adsorbance of the aromatic ring structures associated with the complex carbon compounds.

Protein Removal Experiments

Ten milliliters of fetal bovine serum (FBS) or 10 g. of egg albumin were added to 1 liter of tap water and the solution was passed through the coated or uncoated sand columns. Inffluent and effluent samples were assayed for total

proteins by a commercial kit that utilizes the Lowry procedure (Sigma chemicals, St Luis, MS).

Metal Analysis

Column inffluents and effluents and batch adsorption aliquots were prefiltered through a 0.22 µm filter (Filterite, Timonium, Mr). The solutions were then analyzed by Inductive Coupled Argon Plasma Spectroscopy at the University of Florida Analytical Research Laboratory or at the GRU Kanapaha Water Treatment Facility, Gainesville, FL.

Sand Metal Content Analysis

Sand samples from the columns were taken before and after challenge experiments, and throughout the test period. These samples were analyzed for their metal content after acid digestion. The digestion of the sand coating performed as follows: Five grams of sand was placed in 20 ml. of 6 N hydrochloric acid. The solution was agitated overnight at 37°C. A 1/10 dilution of this solution was made into deionized water and the sample was analyzed by Inductive Coupled Argon Plasma Spectroscopy at the University of Florida Analytical Research Laboratory.

Phosphate and Nitrate Analysis

Phosphate was analyzed by the standard methods (APHA et al., 1992). Orthophosphate was the major phosphate species present. Nitrates were assayed using a Dionex Ion Chromatograph (Model 4500i).

Statistical Analysis

Standard deviations, slopes, correlation and general t test probabilities were determined using PSI-Plot software (Poly Software International, Salt Lake City, Utah)

Results

The coated sand that was initially placed in the columns contained about 1.4 mg Fe and 3.6 mg Al per gram of sand. The amount of metal remained relatively constant throughout the passage of 400 liters of water. Scanning electron microscopy of the coated sand revealed a continuous coating on the surface of the sand. Elemental microprobe X ray analysis revealed the presence of both aluminum and iron on the surface of the sand.

When the columns containing modified sand were exposed to tap water containing combinations of the added metals, they removed 52% of barium, 99% of chromium, 97% of cobalt, 90% of copper, 99% of cadmium, 45% of iron, 99% of lead, 93% of manganese, 65% of nickel, 20% of selenium, and 98% of zinc (Table 17). Columns containing uncoated sand removed less than 50% of any of the challenging metals. When a simulated sewage solution containing various concentrations of these chemical contaminants was passed through the columns, then removals varied from a low 10% to a high of 99.5%. Phosphates were removed by 95 %.The column adsorptive efficiency did not change significantly after 10 or 42 days of exposure to chlorinated tap water (Figure 15).

Columns containing coated sand removed 78% of total proteins when a 1% fetal bovine solution was passed through the column. When a 1% albumin solution was used, 73% removals were obtained (data not shown). When Lake Alice water (UV 254 adsorbance: 0.355; TOC 3.55) was passed through the columns, the UV 254 absorbance was reduced by 35% and the dissolved organic carbon concentrations was reduced to half it original value.

The amount of metal adsorbed onto the surface of the sand before and after passing simulated sewage solutions (containing varying metal concentrations) is shown in Table 20. Also shown, is the amount of metal on the surface of the sand after flushing the columns with 20 liters of water daily for seven days. It can be seen that the adsorbed metals were not released by the tap water.

Results from batch experiments are shown in Table 21. The removal of the tested metals varied, but overall were higher than the column percent removals. Coated sand removed substantially more chemical contaminants than plain sand. Calcium, Lead . cadmium, molibdate, phosphate, and zinc were removed by greater than 99% from water. The removal of these metals was slightly lower when the batch removals were performed in simulated sewage. At best, untreated sand removed an average of 50%. An exception to that would be the removal of lead by untreated sand. Reductions approximating 95% were obtained in those instances.

Discussion

The use of activated carbon, low cost carbons (lignite, bitumen carbons etc.), zeolites and other silicates, and iron oxides in adsorption/ desorption processes has been and still is one of the most largely used methods for the elimination of heavy metals from industrial wastewater. The removal of Pb(II), Ni(II), and Cd(II) individually from waste solutions and industrial waters by adsorption onto waste Fe(III)/Cr(III) hydroxide have recently been studied (Nasmasivayam and Ranganathan, 1995a, b). These studies reported on the efficient removal of the heavy metals from distilled water. The adsorption of the metals from waste water is more complicated, due to the presence of organic ligands that may form soluble complexes with metals and effect the adsorption of metals onto the adsorbents. Therefore, in this study we tested the removal of metals from water and simulated sewage solutions by columns containing metal hydroxide coated sand. In the previous chapters, we examined and reported on the enhanced removal of microorganisms by metallic hydroxide coated solids. The work discussed in this chapter, evaluates the use of iron and aluminum coated solids for the elimination of metal ions and other chemical species from aqueous effluents.

Columns containing 35 mesh coated sand, removed barium, chromium, cobalt, copper, cadmium, lead, manganese, and zinc from water or simulated sewage almost four folds better than columns containing uncoated (plain) 35 mesh sand. The coated columns also demonstrated better iron and nickel

removals from water than columns containing uncoated sand. These columns remained effective for several weeks. The metals that adsorbed to the coated sand in the columns were not subsequently detected in the column's effluent when they were rinsed with deionized water. Alternatively, the adsorbed metals could be detected on the surface of the sand after their adsorption. Their detection could be achieved by ICAP analysis following acid digestion of the coatings or by elemental microprobe analysis (or EDS) using a Scanning electron microscope. Approximations of the adsorptive capacity of the coated 35 mesh sand were derived for some of the various contaminants studied.

In conclusion, solids coated with metallic hydroxides may provide a cost effective efficient means for removing a variety of metallic contaminants from industrial and other effluents. These solids would provide the conveyance of a flow through system and the efficiency of the flocculation procedure. Many other chemical species are found in water treatment effluents. The presence of these species is undesirable and is a source of much concern. Examples of such contaminants include: Chloramines, Trihalomethanes, humics, flavics, and color causing compounds. We are currently investigating the efficiency of removing these harmful or undesirable contaminants from water streams. The initial results seem very promising, but much more research is still needed in this area.

Table 16. The composition of synthetic waste water used in column and batch challenge tests (mg/l).

Organic Substrates:		$N[NH_4Cl]$:	18	Cu[CuCl2.2H2O]:	0.1
Glucose:	300	$Ca[CaCl_2.2H_2O]$:	5	Zn[Zn(Ac)2.H2O]:	0.1
Peptone:	50	K[KCL]:	20	Co[CoCl2.6H2O]:	0.1
Added Salts:		$Mg[MgSO_4.7H_2O]$:	10	Fe[FeCl3.6H2O]:	0.1
$NaHCO_3$:	260	$P[K_2HPO_4]$:	10	Mn[MnSO4.H2O]:	0.1

Source: adapted from Liu et al (1998).

Table 17. Removal of chemical contaminants from water or simulated sewage by columns containing coated or uncoated sand.

Metal Ion (concentration*)	Percent Removal from Tap Water		Percent Removal from Simulated Sewage	
	Uncoated	Coated	Uncoated	Coated
Barium (1 ppm)	9±2	52±2	11±2	50±3
Cadmium (6 ppm)	16±3	99±0.4	17±2	96±2
Chromium(3 ppm)	14±2	99±0.2	14±4	98±0.5
Cobalt (500ppb)	12±2	98±1	13±3	98±0.8
Copper (7 ppm)	11±3	90±2	15±5	85±5
Lead (5 ppm)	23±2	99±0.2	27±5	88±6
Manganese (1 ppm)	11±5	93±6	9±3	92±3
Mercury (200 ppb)	17±4	92±5	-	-
Nickel (11 ppm)	12±2	65±4	13±3	60±5
Phosphate (16 ppm)	9±2	99.1±0.5	12±2	95±3
Strontium (800 ppb)	10±3	64±7	12±2	63±4
Zinc (5 ppm)	13±3	98±1	16±1	94±5

* the average approximate initial challenge concentration is reported.

Note: four liters of tap water containing the indicated concentrations of metals was passed (1 l/min) through columns (25 x 5 cm) containing coated or uncoated 35 mesh sand.

Table 20. The metal content of sand at the beginning, 1 day, and 1 week after challenge with simulated sewage containing salts of the indicated metals.

	Metal content of the coated sand (mg metal/ 50g sand)		
Metallic Species	Day 1(Initial)[1]	Day 2 [2]	Day 7 [3]
Fe	70 ± 5	78 ± 7	72 ± 6
Al	180 ± 13	183 ± 12	185 ± 17
Ba	0.02 ± 0.01	0.54 ± 0.05	0.49 ± 0.09
Cd	0.04 ± 0.003	0.18 ± 0.05	0.19 ± 0.05
Pb	0.28 ± 0.02	0.78 ± 0.04	0.76 ± 0.07
P	0.01 ± 0.00	2.30 ± 0.21	2.62 ± 0.23
Mn	0.16 ± 0.03	0.31 ± 0.07	0.29 ± 0.04
Mo	0.00 ± 0.00	0.87 ± 0.07	0.84 ± 0.03
Zn	0.06 ± 00.02	0.15 ± 0.03	0.16 ± 0.03

[1] The metal content of the sand before the challenge with the simulated sewage containing the metallic salts.

[2] This represents the metal content of the sand after the passage of simulated sewage solutions containing various indicated metals.

[3] After the initial challenge with the simulated sewage solution, 20 liters of water were passed through the columns daily. At day seven the metal content of the sand from the column was determined.

Table 21. Batch removal of chemical contaminants from water or simulated sewage by coated or uncoated sand.

Chemical Species*	Percent Removal from Water		Percent Removal from Simulated Sewage	
	Coated	Uncoated	Coated	Uncoated
Cadmium (8 ppm)	99±0.4	60±6	98±1	42±6
Calcium (2 ppm)	>99	3±1	75±6	2±1
Copper (6 ppm)	95±2	40±2	90±4	47±3
Lead (14 ppm)	99.3±0.2	92±5	99.5±0.4	95±3
Molybdate (14 ppm)	>99.9	10±2	93±2	3±1
Phosphate (10 ppm)	>99.9	48±4	99±0.2	33±4
Zinc (8 ppm)	98±0.8	50±4	95±3	80±3

Note: Only one of each of the challenge chemicals was added to 20 ml of water or simulated sewage containing coated or uncoated sand, and the percent removal was determined and is reported for that chemical species alone.

* the average approximate initial challenge concentration is reported.

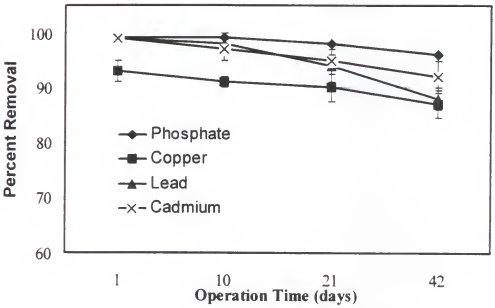

Figure 15. The removal of chemical contaminants from water by columns containing coated sand.

Note: four liters of tap water containing the about 10 ppm of the indicated metals was passed (1 l/min) through columns (25 x 5 cm) containing iron and aluminum (hydr)oxide coated 35 mesh sand.

CHAPTER 6
SUMMARY AND CONCLUSION

Solids such as sand and diatomaceous earth were efficiently coated with precipitates of metallic hydroxides. A new coating process was developed that generated solids that demonstrated enhanced coating durability and improved filtration efficiency. The new coating process utilized the principles of the original in situ precipitation coating process. According to the new coating process, ferric and aluminum hydroxide coatings were deposited on various silica particulates. The deposition was more continuous than that of previous coating processes. This coating proved to be stable and insoluble to subsequent washes. The coatings substantially raised the zeta potential of the solids, therefore imparting a positive charge to their surface.

Solids coated with metallic hydroxides adsorbed microorganisms efficiently from water. These solids raised the filtration efficiency of the many filter systems they were used in. The solids were successfully incorporated into column filters, swimming pool filters, and tap water facet filters. The coated solids removed a variety of enteric viruses, bacteria, and *cryptosporadium parvum* several folds better than uncoated solids. Both molecular and cell culture methods were employed and developed for the efficient detection of the various tested pathogens. The coated solids remained effective for extended periods of time. The length of effective life of the coating depends on the biological and chemical quality of the challenge water.

The presence of sodium or magnesium or aluminum salt solution has varying effects on viral adsorption to various solids. The adsorption of viruses to coated or uncoated diatomaceous earth and to microporous filters is greatly influenced by the presence of varying salt concentrations. These salts can promote or interfere or have no effect on viral adsorption. The effect and its extent depends on the ionic strength, the valency of the salt, the chemical make-up of the solid, the pH, and the amount of organics present. In general, salts interfere with the electrostatic interactions and enhance hydrophobic interactions On the other hand, urea or neutral detergents (Tween 80, pH 7) interfere with the hydrophobic interactions. The presence of both Urea (or Tween 80) and multivalent salt is sufficient to overcome most of the forces responsible for viral adsorption to the microporous filter surfaces.

Coated solids also removed a variety of chemical contaminants from tap water or simulated sewage solutions. Columns containing these solids removed most heavy metals by greater than 90%. They also efficiently removed nickel, mercury, and silver. The solids maintained their removal efficiencies for several weeks and did not lose significant amounts of the metal on their surface after extended use. In addition, the coated solids adsorbed phosphates, dissolved organic carbons and complex non organic materials from eutrophic lake water. At all sampling times, column effluents contained less than 0.01 ppm of iron and aluminum.

In order to expedite the coating of the particles, a rapid and cost effective coating technique and apparatus was designed and developed. This technique

generated coatings of consistent properties and continuous surface coverage on sand and diatomaceous earth. According to this method, solids can be coated in under 20 minutes.

Filters made with solids modified with ferric and aluminum hydroxides seem very promising for use in water treatment processes. The increased pathogen removal efficiency of these solids might reduce the need for the use of potentially harmful disinfectants. Filters containing these solids have great potential for use as point of use filters. Units containing coated filter media would prove to be beneficial in areas were water quality is compromised or where supplies might be contaminated.

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BIOGRAPHICAL SKETCH

Jerzy Lukasik was born on January 22, 1971, in Warsaw, Poland. In 1988 he graduated Al-Jamil High School in Kuwait. He graduated from the Department of Microbiology and Cell Science at the University of Florida with a B.S. degree in 1993 with high honors. Subsequently, he attended graduate school under the supervision of Dr. Samuel R. Farrah of the Department of Microbiology and Cell Science. He completed the requirements for his Master of Science degree in the spring of 1997. After, he continued his research in the same field and subject. He will be graduating with his PhD at the end of the fall semester of 1998.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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